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A novel multimodal model to evoke and modulate human experimental rectosigmoid pain

psychophysical and neurophysiological studies

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Publication date:
2009

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Brock, C. (2009). *A novel multimodal model to evoke and modulate human experimental rectosigmoid pain: psychophysical and neurophysiological studies*. Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University.

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**A Novel Multimodal Model to Evoke and
Modulate Human Experimental Rectosigmoid Pain
Psychophysical and Neurophysiological Studies**

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ISBN (print edition): 978-87-7094-025-2

ISBN (electronic version): 978-87-7094-026-9

The present thesis is partly based on the papers below, which are referred to in the text by Roman numerals. The studies have been carried out in the period from 2006 to 2009 at 1) Mech-Sense, Department of Gastroenterology, Aalborg Hospital, Århus University Hospital & 2) Center for Sensory-Motor Interactions (SMI), Department of Health Sciences and Technology, Aalborg University.

- I:** Brock C, Nissen TD, Gravesen FH, Frøkjær JB, Omar H, Gale J, Gregersen H, Svendsen O, Drewes AM. Multimodal Sensory Testing of the Rectum and Rectosigmoid: Development and Reproducibility of a New Method. *Neurogastroenterol Motil.* 2008;20: 908-18.
doi:10.1111/j.1365-2982.2008.01126.x

- II:** Brock C, Andresen T, Frøkjær JB, Gale J, Olesen AE, Arendt-Nielsen L, Drewes AM. Central Pain Mechanisms Following Combined Acid and Capsaicin Perfusion of the Human Oesophagus. *Eur J Pain.* 2009 in press
doi: 10.1016/j.ejpain.2009.05.013

- III:** Brock C, Olesen SS, Arendt-Nielsen L, Drewes AM. Brain Activity in Rectosigmoid Pain: Real time Estimation of Inhibitory Pathways (*submitted to Pain*)

- *To Marcus & Therese* -

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Acknowledgements

This Ph.D. thesis is based on experimental investigations carried out from 2005 to 2008, during my employment at Mech-Sense at the Department of Medical Gastroenterology, Aalborg Hospital, Århus University Hospital. The experiments were carried out in collaboration with Centre for Sensory-Motor Interactions (SMI), Department of Health Science and Technology, Aalborg University.

I owe my most sincerely gratitude to my main supervisor Professor MD., Ph.D., DMSc., Asbjørn Mohr Drewes, for outstanding inspiration, encouragement, supervision and positive criticism of the research projects and manuscripts. Furthermore I want to express my thankfulness to my two other supervisors Professor MD., DMSc., MPM Hans Gregersen and Professor M.Sc., Ph.D., DMSc Lars Arendt-Nielsen, who have provided excellent research conditions and have contributed to my scientific work in any aspect including advice, inspiration and fruitful discussions.

I want to express my appreciation to MD., Ph.D. Jens Brøndum Frøkjær, who despite his own full calendar and professional carrier, found time to help me in the initial phase. My creative colleague, M. Sc. Flemming Graversen helped me massive with both probe development and initial data extraction.

I am much obliged to the three colleagues: DVM Thomas Dahl Nissen, M.Sc.Pharm Trine Andresen and MD Søren Schou Olesen. The working environment in the lab would not have been the same without your effort and professionalism.

I also want to emphasize the terrific collaboration and the beneficial discussions I have had with M.H Sc Sofie Gry Pristed and M.Sc.Pharm Anne Estrup Olesen, which I have appreciated very much. I am indepted to M. Sc. Carina Gravesen, M. Sc Peter Kunwald and M. Sc. Dina Lelic who helped me continually with technical support.

For practical assistance during experiments and logistics regarding recruitment of healthy volunteers, I want to thank the superb Mech-Sense research nurses Birgit Koch-Henriksen and Isabelle M. Larsen, for their never lacking support, optimism and helpfulness.

M.Sc.Pharm Camilla Staahl, MD Anne L Krarup, technician Anne Brokjær and MD Hans Linde Nielsen have contributed to a wonderful positive working atmosphere. Britta Lund and Susanne Nielsen Lundis are thanked for secretarial help.

M.Sc.Pharm, Ph.D. Jeremy Gale, Pfizer Ltd., is thanked for inspiration, linguistic revision and support during the developmental and writing phase of study II.

I have a special thank to all the volunteers who participated in these comprehensive experiments, without their contribution this research project would not have been possible.

I will express my gratitude towards Aalborg Kommune, especially Bent Jørgensen and Majbrit Odgaard Nielsen, who were enthusiastic and made this employment possible.

The work has received financial support from The Danish Health Research Council (SSVF), The Research Council of North Jutland County, The Research Initiative at Aarhus University Hospital, “Det Obelske Familiefond”, “SparNord Fonden” and Pfizer Ltd. All contributions have been of great value.

Last but not least, I want to thank my family, especially my husband Paul and my children Marcus & Therese, for their never failing support and encouragements.

Christina Brock; July 22nd 2009, Aalborg, Denmark.

List of abbreviations:

ACC:	Anterior cingulate cortex
AMPA:	α -Amino-3-hydroxy-5-Methyl-isoxazol-4-Propionic Acid
ANS:	Autonomous nervous system
AUC:	The area under the curve
CEP:	Cortical evoked potential
CNS:	Central Nervous System
CS:	Central sensitization
DNIC:	Diffuse noxious inhibitory system
DRN:	Dorsal reticular nucleus
EEG:	Electro-encephalogram
ENS:	Enteric nervous system
FEM:	Finite element model
f-MRI:	Functional magnetic resonance imaging
GI:	Gastrointestinal
IBS:	Irritable bowel syndrome
N1; N2	First and second negative peak in an evoked potential
NMDA:	N-Methyl-D-Aspartate
MMP:	Multichannel matching pursuit
P1; P2	First and second positive peak in an evoked potential
PAG:	Periaqueductal grey
PFC:	Prefrontal cortex
RVM:	Rostro ventromedial medulla

I: Introduction

1.1 Visceral pain

Abdominal pain is very common in the general population and pain is the most prevalent symptom in the gastrointestinal (GI) clinic (Russo et al. 2004; Sandler et al. 2000). Both organic and functional diseases of the GI tract give frequently rise to deep pain and they are often difficult to diagnose and treat. Consequently, characterization of visceral pain is one of the most important issues in the diagnosis and assessment of organ dysfunction. The different diseases which give rise to GI pain are often difficult to diagnose and the clinical picture is frequently blurred by co-existing symptoms. This is partly explained by the sparse and diffuse termination of GI afferents at the spinal level of the central nervous system (CNS), but also through the interaction between GI afferents and the somatic, autonomic and enteric nervous systems (ANS and ENS). Hence, complaints related to the ANS and symptoms related to referred somatic pain areas, such as muscles and skin, can easily change the clinical impression of the patient. Hence, to understand the basic neurophysiologic mechanisms, which underlie GI pain, it is important to obtain more knowledge of the visceral sensory system, under standardized experimental conditions (Bochus 1985; Giamberardino 1999).

1.2 Experimental pain

The sources of information regarding GI pain originate from the following four groups of investigators:

1. Animal experiments
2. Experimental pain studies (volunteers and patients)
3. Observational studies in the clinic
4. Interventional studies in the clinic.

As individual sources of information, each of them is inadequate and limited by several biases (Andersen et al. 2000; Arendt-Nielsen 1997; Drewes et al. 2003). Different experimental animal models have been used, and the advantages of these models are obvious: neuronal activity can be studied directly in anesthetized or spinalized animals with invasive recording techniques or via assessment of behaviour (Le Bars et al. 2001; Sengupta & Gebhart 1994). However, as

neurobiology of the pain system differs substantially even between animal species, translations from animal studies to human pain studies have some major shortcomings. Clinical studies are on the other hand limited by the heterogeneity and complexity of diseases even in patients with rather straightforward organic illness (such as acute appendicitis). This is partly caused by different degrees of involvement of either the autonomous nervous system (ANS), the extent of sensitisation, activation of diffuse noxious inhibitory control (DNIC) or a combination of these mechanisms. Therefore, these observational and interventional studies are often too complex to draw any firm conclusions from. For those limitations, human experimental pain models have gained much interest during recent years. In man, pain is closely related to culture, linguistic terms and expressions; hence pain should be regarded as the net effect of complex multidimensional mechanisms involving most parts of the CNS including intensity coding, affective, behavioural and cognitive components. The complexity explains some of the difficulties and challenges in quantifying human sensory experiences with simple neurophysiologic and/or behavioural methods. As a result more advanced human experimental pain studies have increased rapidly during the last decade (Curatolo et al. 2000; Drewes et al. 2003). The ultimate goal of advanced human experimental pain research is to obtain a better understanding of pain mechanisms involved in pain transduction, transmission, and perception under normal and pathophysiological conditions, such as clinical pain.

Human experimental *somatic* models include differentiated stimulation of skin (superficial pain) and muscles (deep pain). The models are highly developed primarily because they are easy to apply. Multimodal stimulation of skin includes mechanical, thermal, electrical and chemical stimulations have all been evaluated within our group (Stahl & Drewes 2004). Usually, the experimental models are divided into methods without (endogenous) and with (exogenous) external stimuli. Endogenous models include ischemic and exercise induced pain, whereas exogenous models employ mechanical, electrical or chemical stimulation (Graven-Nielsen & Arendt-Nielsen 2003).

In order to establish experimental visceral pain, invasive and more comprehensive models are needed. Obviously the risk of perforation and the increased autonomic responses during invasive procedures limits the testing possibilities within the gut. Due to the difficult accessibility of the GI tract, visceral experimental pain testing is far more resource-intensive and challenging than the more traditional somatic pain stimulations. As a result, most previous visceral studies have relied on relatively simple mechanical or electrical stimuli. These methods are easy to apply, but they have numerous limitations (Drewes et al. 2003). As pain is a multidimensional perception, the response

to a single stimulus of a given modality only represents a limited fraction of the entire pain experience. Hence, the possibility of combining different gut stimuli (multimodal stimulation) provides the possibility to more closely imitate the clinical situation and provide extensive and differentiated information on the visceral nociceptive system (Brock et al. 2009; Drewes & Gregersen 2006; Drewes et al 2003). Under these circumstances the investigator controls the experimentally induced pain (including the nature, location, intensity, frequency and duration of the stimulus), the modulation (induction of sensitization, DNIC, or both) and provides quantitative measures of the sensory assessment and the neurophysiologic brain responses (Andersen et al. 2000; Arendt-Nielsen 1997; Rössel et al. 2003). Consequently, the obtained knowledge is used to explain the underlying pain-mechanisms in visceral pain.

1.3 Aims of the thesis

As stated above, there is an ongoing need to develop and test human visceral pain models. Based on this information, development of treatment strategies for visceral discomfort and pain can be done. Under normal circumstances, rectal stimulation evokes numerous sensations: including filling and non-painful urge to defecate. As deformation increases involving wall tension, stress and strain, the feeling changes to an unpleasant and painful urge to defecate.

Altered rectal compliance, anorectal sensations, or both have been proposed as biological markers in functional disorders such as irritable bowel syndrome (IBS) in which enhanced rectal sensitivity has been observed (Mertz et al. 1995; Van, V et al 2008). On the contrary, rectal sensation may be reduced in constipation (Gladman et al. 2005). Previous rectal pain models have not included multimodal stimulation and thus we found it relevant to study the rectosigmoid in order to explore diseases of the large intestine. A validated multimodal approach to this organ is highly warranted.

Hence, the specific aims of the papers behind this thesis were:

- 1) To develop a multimodal rectal probe combining mechanical, electrical and thermal stimulation, and to test the reproducibility of the pain responses to the different modalities (study I).
- 2) To investigate sensitization following a chemical perfusion of the oesophagus. Sensory assessments were done eight cm proximal to the perfusion site as a proxy of secondary

hyperalgesia/allodynia and in the rectosigmoid and rectum to investigate sensory manifestations as a result of distant viscerovisceral convergence (study II).

3) To analyse evoked brain potentials in terms of latency and amplitudes before and after sensitization or induction of DNIC (study II + III).

4) To analyse the electrical brain activity, based on dipolar source locations to painful rectosigmoid stimuli before and after activation of DNIC (study III).

II: Functional neuroanatomy of the peripheral visceral pain system

2.1. Visceral afferents

Visceral afferents mediating conscious sensations run predominantly together with sympathetic nerves reaching the CNS, although some afferents join parasympathetic and parallel pathways. However, the upper oesophagus and rectum also possesses somatic innervations. The importance of this dual innervation is not clear, although rectum has more complex functions than most other viscera and may need differentiated innervations. The peritoneum and parietal serous membranes of the lungs and heart possess their own parietal nerve supply, which is organized like the skin (Bonica 1990). Hence, pain from these structures gives a distinct, intense and localized pain, which is comparable to the pain evoked by skin lesions.

The GI tract has a complex innervation with sensory neurones (extrinsic afferents), and it has its own integrated enteric nervous system (ENS), which project locally. This rich network of neurones and interneurons has a structural complexity and functional heterogeneity similar to that of the central nervous system. It mainly regulates local functions and reflexes such as secretion, motility, mucosal transport and blood flow (Costa & Brookes 1994; Gershon 1981). Motor neurones located within the ganglia of the ENS coordinate these functions largely by regulation from local sensory neurones, although some also receive inputs from the CNS via autonomic (both sympathetic & parasympathetic) pathways (Aziz & Thompson 1998). Although the majority of enteric afferents axons are confined to the gut wall, some can project to the pre-vertebral ganglia of the sympathetic nervous system (Janig 1988), see figure 1.

2.2 Vagal afferent neurones

Fibres travelling with the parasympathetic system project either via the vagal nerve to the brainstem (from the upper gut to the right side of the colon) or via the pelvic nerve to the sacral part of the spinal cord (from the left side of the colon and rectum) (Roman & Gonella 1987). Between 70-90% of the vagal fibres are unmyelinated C-fibre neurones with their cell bodies located in the nodose ganglia situated just below the jugular foramen (Khurana & Petras 1991). Around 80-85% of the vagal nerve fibres are afferents which projects viscerotopically to the medial division of the nucleus of the solitary tract. Vagal afferents are classically believed to mediate non-noxious physiological sensations such as satiety and nausea due to their low response thresholds and saturation characteristics that are within the physiological range (Andrews & Sanger 2002; Berthoud et al. 2002; Sengupta et al. 1989). However, animal experiments have suggested that vagal

afferents may be involved in the central inhibitory modulation of pain. For instance, electrical stimulation of cervical vagal afferents inhibits the responsiveness of spinothalamic tract neurones to noxious stimuli (Ren et al.1991).

Figure 1

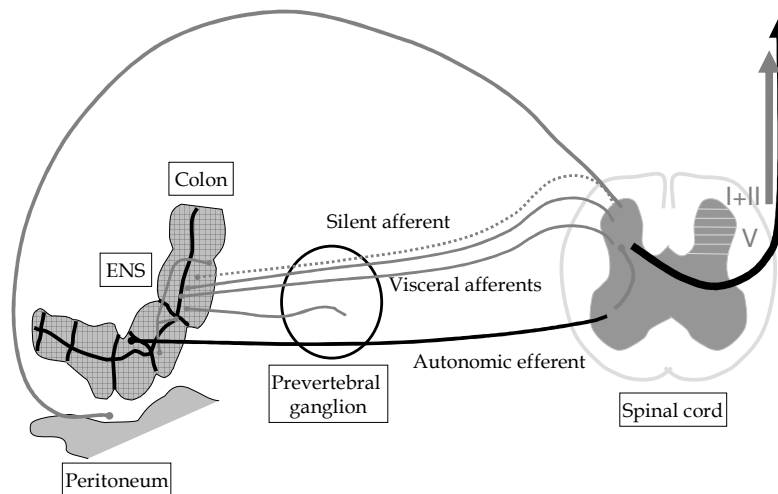


Figure 1: A schematic drawing of the afferent nerve supply of the gut. “True” visceral afferents innervate the gut and run temporarily together with either the sympathetic or parasympathetic nerves to enter the spinal cord. During alterations such as inflammation, “silent afferents” (dashed line) may become activated and contribute to the sensory response. The peritoneum and parietal serous membranes of the lungs and heart has its own parietal nerve supply, which is organized topographically like the somatic structures.

2.3 Silent nociceptors

The GI afferents have been characterised by different techniques and much controversies exist. Unlike cutaneous pain where the existence of specific nociceptors is documented (Cervero 1988; Torebjork 1985), most visceral nociceptors are probably non-specific (polymodal) and respond to different stimuli being for example mechanical, thermal and chemical (Cervero 1994; Sengupta & Gebhart 1994; Su & Gebhart 1998). A special subset of the nociceptors is “silent” nociceptors, which become active during inflammation. Silent nociceptors have mainly been demonstrated in the bladder and rectum where they constitute up to 50% of the afferent inflow during inflammation

(Sengupta & Gebhart 1994). We believe that the chemical perfusion (acid+capsaicin), which was used in study II may activate such silent receptors.

III: Central pain processing

3.1 The dorsal horn neurones

From the cell bodies within the dorsal root ganglion, spinal visceral afferents enter the spinal cord and ascend or descend one or two spinal levels in the dorsolateral fasciculus (Lissauer's tract) before terminating within the grey matter, predominantly in lamina I, II and V. To get an overview of the primary ascending tracts, see figure 2. Most of the second order spinothalamic cells in lamina I are nociceptive specific cells, whereas those in lamina V are “wide-dynamic neurons” with graded responses to physiologic as well as noxious stimuli (Craig 2003).

Figure 2

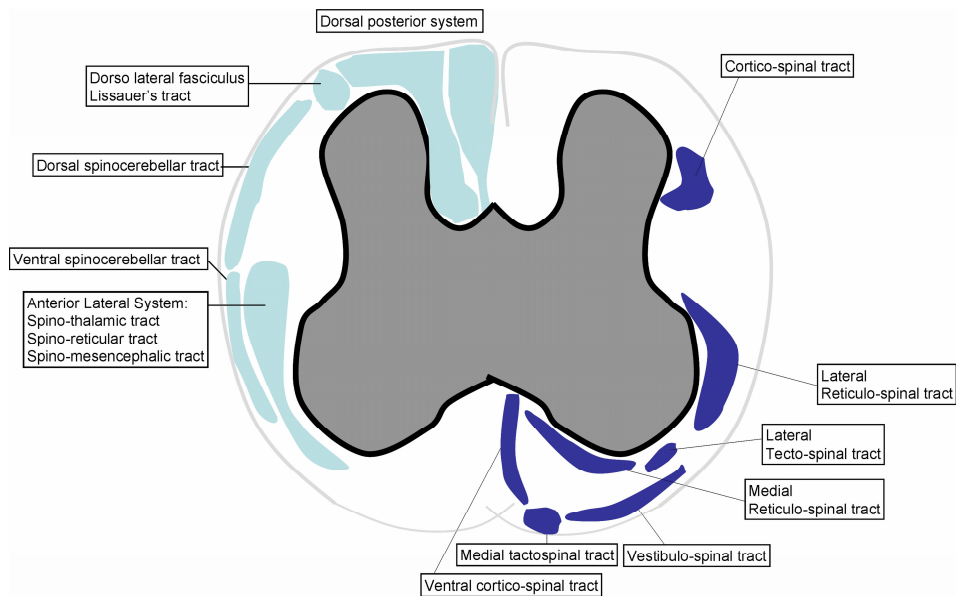


Figure 2: Shows a schematic and simplified section through the spinal cord, and highlights the primary spinal tracts involved in ascending (light blue) and descending (dark blue) information.

In the thoracic spinal cord more than 75% of all dorsal horn neurones receives both somatic and visceral information, which is in contrast to the actual number of GI afferents (5-15% of the inflow) (Cervero 1988; Sengupta & Gebhart 1994). The low density of sensory innervation and diffuse termination may therefore explain why large areas of the gut appear to be relatively insensitive to pain stimuli (Bielefeldt et al. 2005; Cervero 1988). Whereas the somatic afferents have a somatotopic organisation on specific neurons in the spinal cord, the GI innervation is probably much less specific (Cervero & Laird 1999). In laminae I and V, the GI afferents converge on a large

scale with neurones, which also receives input from superficial and deep somatic tissue as well as other viscera (Giamberardino 1999). This explains why only a few GI afferent fibres can activate many neurons through the extensive functional convergence, and this wide activation of the CNS may explain the diffuse and unpleasant nature of GI pain (Giamberardino & Vecchiet 1996). Second order neurones in the afferent pathway have a cell body in the dorsal horn of the spinal cord and relay signals to the brain via a number of ascending tracts.

3.2 Ascending spinal tracts

The ascending spinal tracts that convey sensory information to supraspinal structures are contained within the anterior lateral and posterior tract systems. A schematic drawing is shown in figure 3. The anterior lateral system comprises the spinothalamic, spinoreticular, spinomesencephalic and spino-limbic tracts.

Figure 3

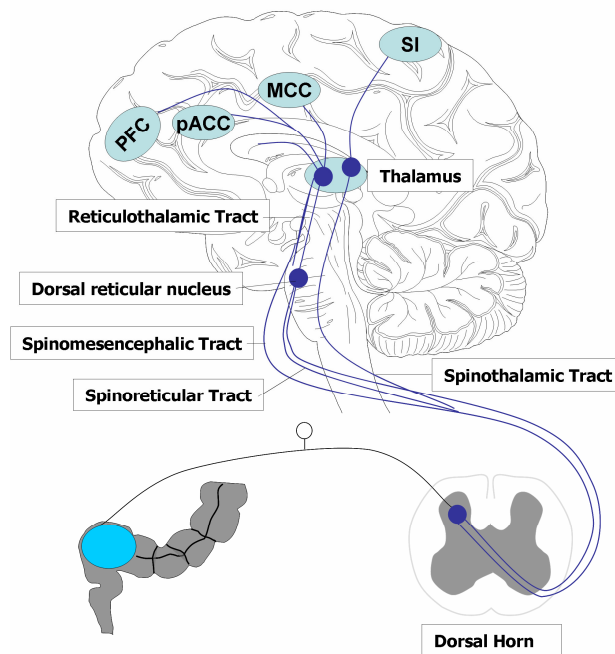


Figure 3: The principal visceral projections from the spinal cord to sub-cortical and cortical structures. The spinothalamic tract terminates in the medial and posterior part of thalamus. Thalamocortical fibres then project to the somatosensory cortex. The spinoreticular tract terminates in the reticular formation of the brainstem. The reticulothalamic tract projects from the dorsal and caudal medullary reticular formation to the medial thalamus. The spinomesencephalic tract projects to various regions in the brainstem, including PAG, locus coeruleus and the dorsal reticular nucleus. Thalamocortical projections from thalamus project to the cingulate cortex and insula, which are involved in processing noxious visceral and somatic information. The brain regions innervated by these pathways that respond to painful visceral stimuli include the thalamus, insula, amygdala and the anterior cingulate cortex. Other pathways such as the dorsal column pathway exist but are not shown. Adapted and modified from (Mertz 2002).

The medial and lateral subdivisions of the spinothalamic tract project to the medial/intralaminar and ventral/ventral posterior lateral nuclei of the thalamus respectively (Ammons et al.1985). Third-order thalamocortical fibres then project to the somatosensory, insula and medial prefrontal cortices (Loewy 1990). The spinothalamic tracts mediate sensations of pain, cold, warmth and touch are also important for sensory discrimination and localisation of visceral and somatic stimuli (Willis, Jr. 1985).

The spinoreticular tract conducts sensory information from the spinal cord to the reticular formation in the brainstem. The reticular formation is mainly involved in the reflexive, affective and motivational properties of such stimulation (Casey 1980). Third-order reticulothalamic tract neurons project from the dorsal and caudal medullary reticular formation to the medial and intralaminar nuclei of the thalamus. From the intralaminar nuclei, ascending pain signals spread bilaterally to the prefrontal cortex, including the anterior cingulate cortex (ACC) (Willis & Westlund 1997). The spinomesencephalic tract ascends the spinal cord with fibres to various regions in the brain stem, including the PAG, locus coeruleus, and dorsal reticular nucleus (DRN) in the medulla (Willis & Westlund 1997). The spinoreticular-thalamic pathways are involved not only in the nociceptive transmission but also in the descending pain control (Monconduit et al. 2002).

The spino-limbic tract project to areas such as the amygdala, medial thalamus, hypothalamus and other limbic structures and are also believed to be important in mediating the motivational aspects of pain (Willis & Westlund 1997).

The posterior system comprises three synapsing tracts: first order dorsal column neurones, the post-synaptic dorsal column pathway and the spinocervical tract. These pathways were not believed to convey nociceptive information; however, recent studies have highlighted the importance of the dorsal column in viscerosensory processing. Al-Chaer demonstrated in primates that the responsiveness of neurones in the ventral posterior lateral nucleus of the thalamus to colorectal distension could be significantly attenuated by dorsal column lesions (Al-Chaer et al. 1996). Lesions of other tracts had no consistent effects thus supporting the role of the dorsal column in conveying visceral nociceptive input to the thalamus.

3.3 Descending spinal tracts

The descending spinal pathways, through which the brain controls the spinal activity via either facilitation or inhibition, includes among others the dorsal cortico-spinal tract, the lateral reticulo-

spinal tract, the medial reticulo-spinal tract and the ventral cortico-spinal tract. A schematic drawing of the descending spinal tract is seen in figure 2.

3.4 Pain processing in the brain

Knowledge of how the brain processes sensory information from visceral structures is still in its infancy; however our understanding has been propelled by technological imaging advances such as functional magnetic resonance imaging (fMRI), magnetoencephalography, positron emission tomography (PET), and electroencephalography (EEG). Human studies have non-invasively demonstrated the complexity of neuronal networks, which are involved in pain processing. Hence, a number of the subcortical and cortical regions, which are involved in the process, are shown in figure 4. The neuronal pathways, which are involved in the perception of visceral pain, are dynamic and amenable to change in response to internal or external stressors. Numerous mechanisms can be engaged in response to stressors along the entire neuraxis: From the primary afferent level right up to the cerebral cortices. These changes induce a high degree of plasticity in the nervous system and the ultimate outcome of pain perception is brought about by a delicate balance between facilitatory and inhibitory mechanisms, (see chapter VI).

3.3.1 The cerebral cortex

The primary and secondary somatosensory cortices (SI and SII respectively) are both involved in processing non-noxious somatosensory information, such as pressure and warmth, providing vital information about the external environment and allowing modulation of motor function. Both regions receive nociceptive input from the thalamus. Nociceptive neurons in both areas are thought to encode the sensory discriminatory aspects of pain processing together with the SII cortex, which is also involved in recognition, learning and memory of painful events (Schnitzler & Ploner 2000). Human case studies support the above as e.g., Ploner et al. have reported a patient with an ischemic lesion of the secondary and primary somatosensory cortices, who was both unable to localise a painful laser stimulus on the affected hand and unable to recognize the nature of the stimulus even when appropriate terms were presented to him (Ploner et al. 1999).

Figure 4

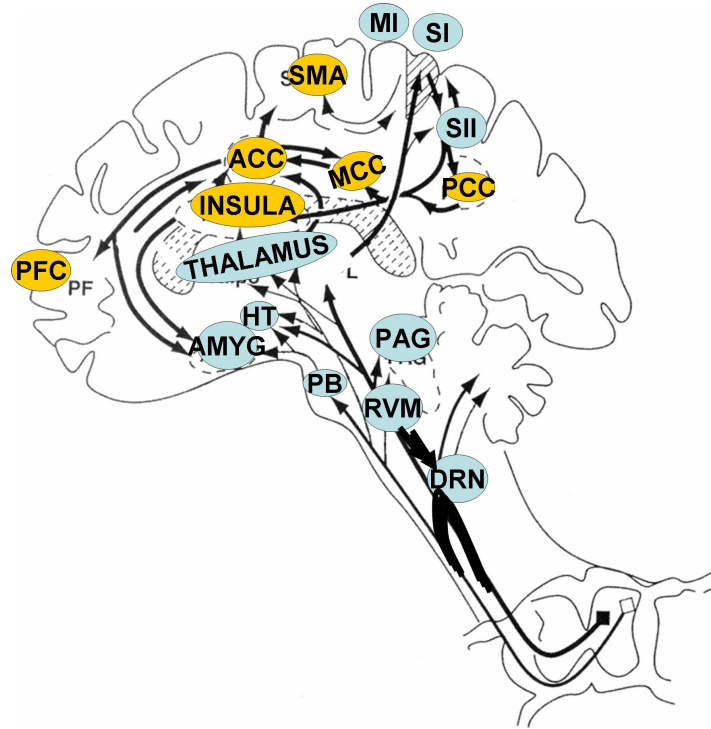


Figure 4: A drawing of the subcortical and cortical structures, which are activated in response to visceral pain. Abbreviations: DRN: dorsal reticular nucleus; RVM: rostroventral medulla; PAG: periaqueductal grey; PB: parabrachial nucleus of the dorsolateral pons; AMYG: amygdale; HT: hypothalamus; ACC: anterior cingulate cortex; MCC: mid cingulate cortex; PPC: posterior parietal complex; SI + SII: primary and secondary somatosensory cortices respectively; MI: motor cortex; SMA: supplementary motor area and PFC: prefrontal cortex; The structures, which are highlighted with orange, are structures in which we found brain activity in study III. Adapted and modified from (Price 2000).

3.3.2 Insula

The insula receives projections from SII and from neurons in the ventromedial posterior nucleus in the medial thalamus, and has been shown to be activated by visceral stimuli (Aziz et al. 2000). It has also been proposed to be involved in autonomic reactions to noxious stimuli and in affective aspects of pain-related learning and memory, but has no role in sensory discrimination (Schnitzler & Ploner 2000).

3.3.3 The cingulate cortex

The cingulate cortex is an extensive area of the limbic system with anterior and posterior regions, the former of which has been implicated in the processing of both visceral and somatic sensation. Two particular areas of the anterior cingulate cortex (ACC) deserve attention: the anterior

midcingulate cortex and the more rostral perigenual part of the cingulate cortex. The midcingulate cortex is believed to be involved in response selection, attention and preparatory motor functions. The perigenual part of the ACC has connections with brainstem autonomic nuclei and is involved in visceromotor control and modulates the autonomic and emotional responses to external stimuli (Devinsky et al. 1995; Vogt et al. 1996). Lesion studies in patients following cingulotomy have shown that whilst pain was still perceived, it was less distressing and there was less motivation to avoid the painful stimulus, confirming the role of the ACC in the affective-motivational aspects of pain processing (Davis et al. 1994; Peyron et al. 2000).

Activation of the prefrontal cortex has also been observed in response to both somatic and visceral sensation. It interacts with the ACC and is believed to be responsible for cognitive evaluation, self-awareness, attention and behavioural control (Frith & Dolan 1996). In study III we found predominant brain activity in the cingulate cortex. Furthermore we found parallel dipoles in the prefrontal cortices, insula and supplementary motor area.

3.3.4 The amygdala

The amygdala is part of the limbic system in the medial temporal lobe which has a role in emotionality, the emotional evaluation of sensory stimuli, emotional learning, memory and affective disorders such as anxiety and depression (Davidson 2002; Gallagher & Schoenbaum 1999; Zald 2003). Emerging data suggest a role for the amygdala in modulating nociception, in particular the link between pain and emotion. Sensory information reaches the amygdala mainly through the lateral and basolateral nuclei of the amygdala.

III: Sensitization

4.1 Peripheral sensitization

Inflammatory mediators such as histamine, bradykinin, serotonin and prostaglandines have experimentally shown to activate and sensitize peripheral terminals of primary afferents (Bueno et al. 1997; Cohen & Perl 1990; Gebhart 2000b; Schaible & Schmidt 1988; Su & Gebhart 1998). These chemicals can alter the synaptic function by modifying either the release of neurotransmitters from presynaptic terminals or transmitter responsiveness on the postsynaptic membrane. Hence, *sensitization* is characterised by:

- 1) Increase of the firing frequency,
- 2) Lowered firing threshold (depolarisation of the nerve cell),
- 3) Enhanced responsiveness (increase in the number and/or amplitude of neuronal discharges),
- 4) Expansion of the receptive field of the neuron.

The same mediators may also recruit “silent nociceptors”, which results in an increased input of nerve signals to second-order neurones and sensitization of the spinal neuron. The pattern of increased activity alters the nociceptive circuits, which may be maintained even after resolution of the peripheral stimulus (Besson 1999; Bueno et al. 2000). This synaptic plasticity allows the nervous system to adapt to adverse stimuli. Depending on the synapse and frequency, intensity and duration of activity, both increasing activity (facilitation, potentiation or sensitisation) and decreasing activity (habituation, depression or desensitization) can be induced (Mendell 1984).

Such peripheral mechanisms have been implicated in animal models of post-injury gut dysfunction. For instance, animal studies in mice with ongoing intestinal contractile dysfunction following resolved gut infection have demonstrated the persistence of local inflammatory mediators such as cyclooxygenase-2 (Barbara et al. 2001; Barbara et al. 1997). Moreover, inflammatory mediators can sensitize- when instilled into the rat colon - the response of pelvic afferent nerve fibres to subsequent colonic distension (Su & Gebhart 1998).

4.2 Central sensitization

At the spinal level, aspartate and glutamate are the prevalent excitatory neurotransmitters (Merighi et al. 1991; Tracey et al. 1991). They are released at the central terminals of primary afferent neurones in conjunction with a number of other neurotransmitters including substance P,

prostaglandin E2 and brain derived neurotrophic factor. The two receptors α -amino-3-hydroxy-5-methyl-isoxazol-4-propionic acid (AMPA) and N-Methyl-D-Aspartate (NMDA) open and close quickly, and are thus responsible for most of the fast excitatory synaptic transmission in the spine. Normal physiologic excitatory transmission is supposed to occur mainly through the AMPA receptor and increased levels of glutamate e.g. due to peripheral sensitisation leads to increased activation. The NMDA receptors are likely to play an important role in mediating the increase in spinal central excitability and possibly also in acute pain of normal viscera (Bueno et al. 2000;Coderre et al. 1993; Giamberardino 1999). Extensive glutamate release result in a removal of the magnesium ion block of the NMDA receptor and its subsequent activation (Woolf & Thompson 1991). Substance P, which binds to the NK1 receptors, affects the postsynaptic membrane. This phenomenon has been termed *central sensitization* (CS) and is believed to be responsible for the pain hypersensitivity that occurs in surrounding healthy tissues (secondary hyperalgesia or allodynia).

Spinal hyper-excitation and convergence leads to altered pain-processing, in which incoming information from visceral nerve afferents converge with spinal neurons that would not normally be activated or activate them more strongly (McMahon et al.1993; Woolf 1993). Hence, CS is characterised by an increased firing frequency and decrease in activation threshold of the dorsal horn neurones; an enhanced response to duration and magnitude of noxious stimuli and an expansion of the mechano sensitive receptive field of dorsal horn neurones (Woolf 1995).

The phenomenon of viscero-visceral *hyperalgesia*, where activation of the pain system in one organ affects sensitivity in distant and otherwise healthy organs, is supported by numerous animal studies, (Bielefeldt et al. 2005; Garrison et al.1992; Giamberardino et al. 2002; Qin et al. 2005). Electrophysiological studies at the spinal cord level in animal models (Giamberardino et al. 1996; Roza et al.1998) support the assumed existence of a central component in the production of referred hyperalgesia from viscera. In a study, hyperexcitability of spinal neurones were observed early in the algogenic process (Roza et al.1998). Ureteric stones caused excitability (decreased threshold) of the spinal neurones which also received convergent input from somatic receptive fields. Furthermore, rats with surgically-induced endometriosis display additional pain behaviour and muscle hyperalgesia following experimental urinary stone implantation (Giamberardino et al. 2002).

Viscero-visceral hyperalgesia has also been shown in humans. Experimental acid-perfusion of the distal oesophagus induced secondary allodynia in the proximal oesophagus (Sarkar et al. 2006).

Recently, proximal oesophageal hyperalgesia resulting from small, repetitive acidic perfusions (resembling clinical gastro-oesophageal reflux) was also shown (Matthews et al. 2008). Moreover, in response to duodenal acidification increased sensitivity to oesophageal electro-stimulation has been demonstrated, and our own group have shown sensitization of sigmoid colon and rectum following oesophageal acidification (Hobson et al. 2004; Frokjaer et al. 2005).

In study II, we explain the measured extra segmental hypersensitivity in the rectum (to heat and mechanical stimulation) following oesophageal perfusion as CS, which lead to viscerovisceral *hyperalgesia*.

4.3 Primary hyperalgesia

Peripheral nociceptor sensitization, which underlies the hyperalgesia that immediately develop around an injury site, is called primary hyperalgesia. Animal studies have shown that peripheral sensitisation caused by tissue injury by e.g., bradykinin, serotonin and substance P resulted in an increased response to a given stimulus and/or an increase in the spontaneous activity of the afferent (Gebhart 1995; Gebhart 2000b). Peripheral inflammation has also been shown to activate “silent nociceptors” (Koltzenburg 1994). The activated fibres develop ongoing activity and display major changes in receptive fields and pattern of referral within minutes after tissue irritation. When a painful sensation is produced by a non-noxious stimulus, the term allodynia is used.

4.4 Secondary hyperalgesia

Central rather than peripheral sensitisation is thought to be accountable for secondary hyperalgesia. In the somatic system it is defined as increased sensations to painful stimuli, which exists in a much larger area than the site of injury. In the GI system referred somatic pain/hyperalgesia is thought to be equivalent to the secondary hyperalgesia observed in the cutaneous system (Coderre et al. 1993; Gebhart 2000; Jänig & Häbler 1995). In the spinal cord nociceptive neurons activates secondary messengers and presynaptic transmitter release, which leads to positive feedback loops and to increased excitability of the dorsal horn neurones (CS).

4.5 Clinical hyperalgesia.

Primary allodynia/hyperalgesia combined with central hyperexcitability can sufficiently explain the pain associated with inflammatory conditions as well as that in functional disorders of

the GI tract. Thus, following peripheral stimulation (such as inflammation due to gastro-oesophageal reflux) subsides, sensitized second order neurones continue to fire, and sub-threshold regulatory stimuli are still perceived as painful. An example from the clinic is seen in patients suffering from irritable bowel syndrome where physiological bowel movements are perceived as painful (Coderre et al.1993; Gebhart 2000b; Kolhekar & Gebhart 1996; Mayer et al. 1995; Willis 1993).

Under some circumstances the central hyperexcitability seems to outlive the presence of the primary focus (Gebhart 2000b; Laird et al.1997). Furthermore it has been shown that 90% of patients who previously suffered from colics due to calculosis of the upper urinary tract 3-10 years earlier, still suffered from hyperalgesia in the somatic tissue (Vecchiet et al.1992). However, the hyperalgesia which is demonstrated in patients suffering from functional GI disorders may also be maintained by other factors which can explain the central hyperexcitability causing lifelong symptoms. Under such circumstances more permanent alterations in GI sensory processing such as those caused by e.g., perinatal events, sexual and verbal abuse, other stressful life events (the so-called psychological hypothesis), genetic differences etc., may co-exist as etiologic factors (Gebhart 2000b; Hu & Talley 1996; Mayer & Gebhart 1994; Rao 1996).

These theories were supported by Al-Chaer et al. who showed that neonatal rats that were separated from their mother, developed spinal hyperexcitability and chronic visceral/deep hyperalgesia following painful colonic irritation (Al Chaer et al. 2000). Thus, the authors hypothesised that transient noxious stimulation in a state where the nervous system is vulnerable is able to cause long-lasting central sensitisation. Abnormalities of central control mechanisms may also contribute to the findings (Mayer & Gebhart 1994). As pain is difficult to control in functional GI diseases, further knowledge on hypersensitivity is crucial.

V: Convergence

5.1 Mechanisms behind viscerosomatic convergence

The suggested theories behind the pathogenesis of *viscero-somatic* convergence goe back more than a century (MacKenzie 1893; Ross 1888; Ruch 1961; Sturge 1883). Today the mechanisms responsible for referred pain areas to adjacent anatomical segment are still not known in details, but although simplified, convergence between visceral and somatic afferents in the spinal cord seems to be of importance for development of referred pain areas, for details see (Arendt-Nielsen et al. 2000). The mechanism is further potentated by means of several molecular processes, where hyper-excitation of the spinal neuron occurs (for more details regarding sensitization: see chapter III). Moreover, brainstem convergence of viscerosomatic input has been observed upon vagal stimulation and pelvic nerves (Hubscher et al. 2004).

It is believed that referred hyperalgesia of somatic tissues is caused by a process of central sensitisation which takes place in the CNS, triggered by the massive afferent visceral barrage. Experimentally it has been shown that repetitive stimulation of the gut or bladder increased the referred pain area in healthy subjects (Ness & Gebhart 1990). The increased pain and referred pain areas to repetitive stimulation could indicate that mechanisms related to central hyper excitability were evoked and thus opened latent connections (Arendt-Nielsen et al. 2000).

A clinical study by Giamberardino et al. showed that patients with visceral pain had structural changes in the areas of pain referral, in which an increased thickness of subcutis and a decreased thickness of muscle were measured by ultrasound (Giamberardino 1999). Another study showed increased blood flow in the referred pain area following intraluminal application of capsaicin in the ileum or colon (Arendt-Nielsen et al. 2008a).

In study I we assessed referred pain areas to rectal multimodal stimulation. However, the somatic referred pain area to rectal stimulation was vulnerable to bias. This is partly because most people find it difficult to quantify referred pain in that anatomical region, but it is further complicated because the subjects shall differentiate referred pain from the feeling of the rectal probe positioned through the anal canal (Frokjaer et al. 2005b). The diffuse localization of the referred pain areas to the multimodal stimulations did not project to a single specific dermatome, which is in consistency with visceral sensory afferents projecting to the spine with a segmental overlap.

In study II, we assessed the oesophageal referred pain areas to electrical stimulation, and found that they were diminished after sensitization. We believe that this finding, in conjunction with hypoalgesia can be interpreted as an activation of descending inhibition.

4.2 Mechanisms behind viscerovisceral convergence

Animal studies have showed that recordings from the feline spinal neurones show convergence of oesophageal and somatic afferents into the same second neuron. In the same study, turpentine-induced inflammation of the distal oesophagus resulted in a decreased threshold of the spinal neurones to oesophageal distension (Garrison et al.1992). These findings confirm that the painful afferent signals are transmitted preferentially along the sympathetic nerves into the spinal cord.

In humans, most of the visceral afferents converge with lamina I, II and V spino-thalamic tract neurons, which receive input from both superficial and deep somatic tissue as well as other viscera (Giamberardino 1999). Most visceral organs exhibit spinal representation overlapping multiple segmental levels (Bielefeldt et al. 2005). Although the neuronal mechanisms are more complex this convergence leads to viscerovisceral hyperalgesia.

4.3 Clinical viscerovisceral convergence

In organic diseases, painful sensations can be explained by increased afferent input from the periphery to the spinal and supraspinal neurones due to ongoing peripheral tissue irritation and neuro-transmitter release (see section 4.1). Longer-lasting or repeated painful stimuli lead to *allodynia* and *hyperalgesia* of the stimulated area. The widespread *convergence* in the spinal cord also leads to spread of the pain and hyperalgesia to uninjured tissue manifested as:

- 1) Referred somatic pain and
- 2) Viscerovisceral hyperalgesia (secondary hyperalgesia)

Normally, these changes will rapidly disappear after the initial stimuli have subsided. However, in patients with chronic abdominal pain, such as the irritable bowel syndrome (IBS), visceral hypersensitivity has been found in the absence of any visceral organic disease (Accarino et al. 1995; Bradette et al. 1994; Lembo et al. 1994; Mertz et al. 1995; Munakata et al. 1997). Viscerovisceral convergence may also explain several co-morbid conditions such as increased number of angina attacks in patients with gallbladder calcinosis, and increased number of painful sensations to normal air and faeces in the gut in patients primarily suffering from dysmenorrhoea (Brinkert et al. 2007; Foreman 1999; Giamberardino 2000; Giamberardino et al. 2001; Ness & Gebhart 1990). Furthermore the frequent airway symptoms in patients suffering from reflux disease may not only be

related to direct aspiration of the gastric refluxate, but also to vasovagal reflex mechanisms evoked by acid-related central hyperalgesia (Fass et al. 2004; Javorkova et al. 2008).

The findings in study II showed *viscero-visceral hypersensitivity* to heat and mechanical stimulation of the rectum following oesophageal chemical perfusion. Simultaneously *hypoalgesia* was observed to electrical stimulation in both the oesophagus and sigmoid colon. The findings reflect complex central mechanisms involved in pain control. Hence the established model in study II may resemble a more realistic model of clinical pain.

VI: Central pain control

6.1 Mechanisms behind pain control

The brain controls the complex networks, which are involved in descending pain control (Fields & Basbaum 1999). However, the underlying mechanisms are complex and not yet fully understood. Animal data have shown that in addition to descending inhibition, the same sites can also cause descending facilitation and hence the subjective pain perception is a dynamic balance of a bidirectional pain-control mechanisms (Ren & Dubner 2002).

Inhibitory mechanisms:

- Direct inhibition of projecting neurons
- Inhibition of excitatory or increased inhibitory transmitter release from primary afferents
- Excitation of inhibitory interneurons through GABA activation

Facilitatory mechanisms:

The descending facilitatory mechanisms are similar to the descending inhibitory mechanisms, but obviously directed opposite.

- Direct hyper-excitability of projecting neurons
- Increased excitatory or decreased inhibitory transmitter release from primary afferents
- Excitation of excitatory interneurons through glutamate activation

6.2 Inhibitory control

Several levels of pain modulating mechanisms contribute the descending inhibitory circuit which results in direct or indirect inhibition of spinal or supraspinal neuronal responses (Le Bars 2002; Millan 2002). We believe that the underlying mechanisms in the inhibitory circuits shall be considered as dynamic and plastic. For simplicity, we have listed four different modulating regions within the central nervous system, which all (alone or in conjunction with each other) contribute to the inhibitory circuits. Hence they may not be considered as independent structures.

1) Inhibition operating primarily at the spinal level (modified gate control), involves spinal interneurons located in the dorsal horn. Only islet cells in lamina I are exclusively inhibitory (Maxwell et al. 2007).

2) DNIC operating through a spino-bulbo-spinal loop involving the dorsal reticular nucleus, which contains multiceptive neurons with the whole body as receptive field (Le Bars 2002; Pud et al. 2009).

3) Inhibition through a brainstem network consisting of PAG-RVM. These structures possess modulating abilities through so called “ON-cells” and “OFF-cells”, which are pro-nociceptive or anti-nociceptive respectively. These supraspinal sites can either enhance or inhibit nociception and the balance between them is dynamic (Calvino & Grilo 2006; Fields et al.1995; Heinricher et al. 2009).

4) Endogenous inhibition involving a pain-matrix consisting of frontal-cortical-limbic-brainstem top-down pathways (Mayer et al. 2005; Price 2000). Activation of the cingulate gyrus is often reported. The structure is believed to links perception and emotion among others (Derbyshire 2000; Peyron et al. 2000).

6.3 Spinal pain control

The modified gate control theory of pain, first put forward by Melzack and Wall in 1962 has been of great importance in understanding the underlying mechanisms of segmental inhibition (Melzack & Wall 1965). It builds on the theory, that large myelinated non-nociceptive A β fibres activates an inhibitory interneuron, which in turn stabilizes the nociceptor and prolongs the period for depolarization of the pain-coding afferent. For a schematic drawing, see figure 5.

Figure 5

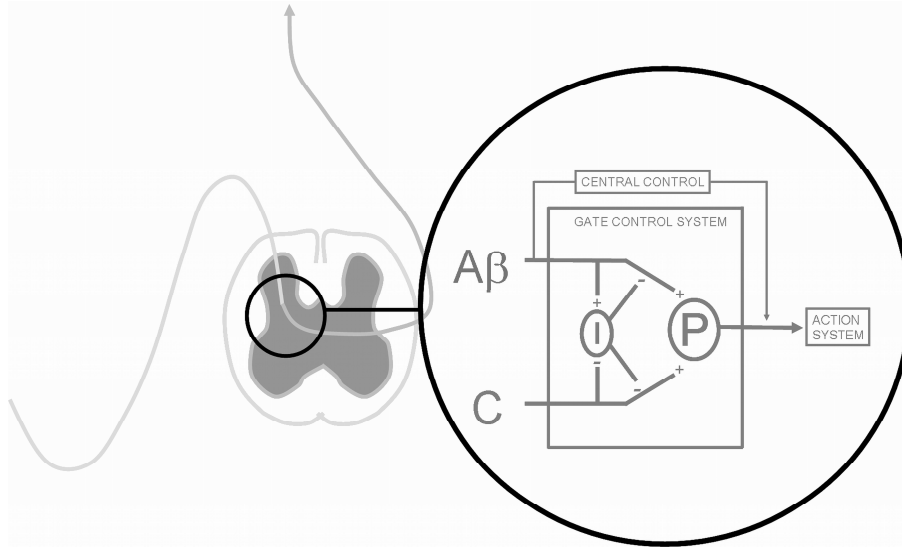


Figure 5: The original gate-control theory, proposed by (Melzack & Wall 1965) suggests that there is a balance between two types of influences exerted on spinal non-specific nociceptive neurones, and their axons constitute the ascending spinothalamic or spinoreticular tract. Modified from (Calvino & Grilo 2006)

Later, a more complex model of spinal pain control was proposed, which took into account that dorsal horn neurones also are modulated through descending inhibitory control from the brainstem. See figure 6, on the following side.

Figure 6

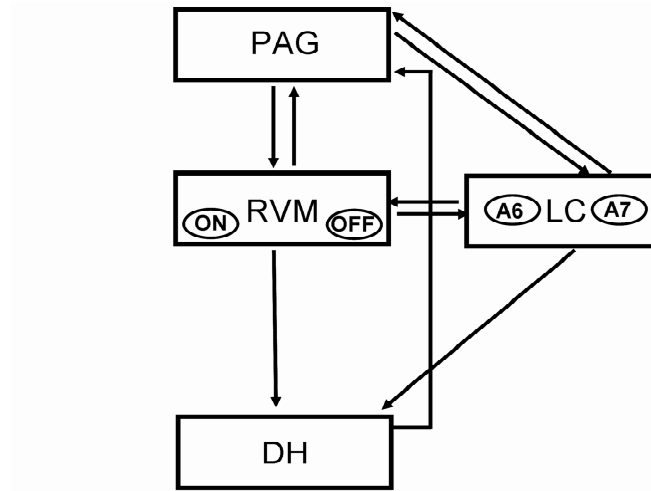


Figure 6: Spinal-medullary-spinal negative feed-back loop underlying an endogenous analgesic system called into play by nociceptive stimuli, as suggested by (Basbaum & Fields 1984). The schematic drawing shows that besides the descending inhibition through the brainstem circuit of RVM-PAG (including the dynamic shift between OFF- and ON cells) adrenergic (A6 and A7) descending inhibition from locus coeruleus co-exist. Abbreviations: DH: dorsal Horn; RVM: rostro-ventral medulla, PAG: periaqueductal grey and LC: Locus coeruleus

Most of the literature regarding descending pain-control derives from animal experimental data. Descending control from primarily PAG-RVM and locus coeruleus modulates the spinal nociception through inhibitory and facilitatory pathways that can be both serotonergic and adrenergic (Calvino & Grilo 2006).

Differentiated descending control on dorsal horn neurons has been proposed, depending on the degree of C-fibre input (Heinricher et al. 2009). As C-fiber input primarily terminates in the superficial layers of the dorsal horn (Lamina I and II), the C-fibre evoked responses in the deep dorsal horn must be received on superficially directed dendrites or relayed via superficial interneurons (Morris et al. 2004). The model can be regarded as an “extension” of the spinal inhibition, see figure 7.

Figure 7

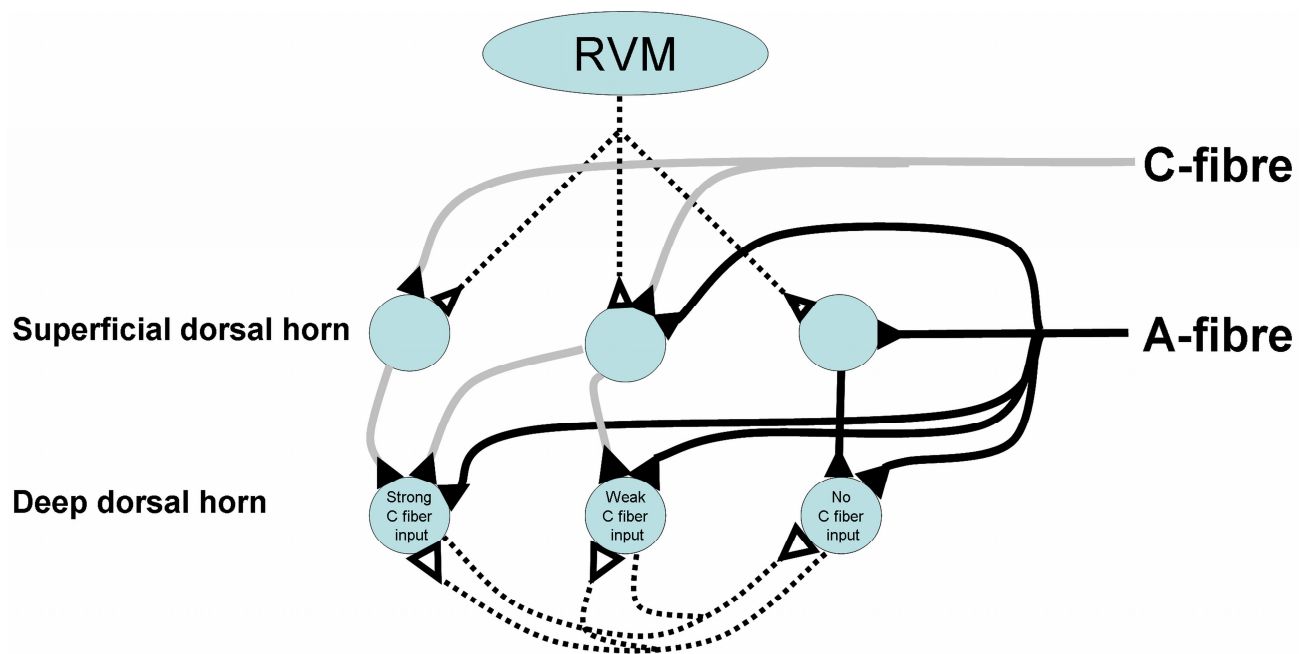


Figure 7: A model to explain how descending control from RVM, which targets different populations of superficial dorsal horn neurons, could produce an inhibition of deep dorsal horn neurons that is proportional to their C-fibre input, but a facilitation of other neurons with either weak or no C-fibre input. Solid lines represents direct (monosynaptic) connections, dashed lines represent indirect (polysynaptic) connections between neurons. The solid triangles represent the excitatory synapses whereas the open triangles represent the inhibitory connections. The RVM inhibits superficial dorsal horn neurons, which relay information carried by C-fibres to the deep dorsal horn. The net inhibitory or facilitatory effect of RVM stimulation is also a function of reciprocal inhibition between neurones with either strong or weak C-fibre input at the segmental level.

Modified from (Heinricher et al 2009).

A-fibre nociceptive input also terminates in the superficial lamina although some input is directly to the deep dorsal horn. Descending modulation pathways terminate heavily in the superficial dorsal horn. Hence, although the activity of deep dorsal horn cells may be influenced directly by descending pathways, much of the descending influence is likely to be secondary to modulation in the superficial dorsal horn (Heinricher et al. 2009).

If the sensitized neurons in study II, primarily activated the extended spinal inhibition, we would have seen that the pain response to heat-stimulus (C-fibre) was inhibited (higher threshold), and electrical stimulus (C-fibre and A fibre) was enhanced (lower threshold). However, our data showed the opposite. Thus we conclude, that more central pain mechanisms were brought into play, e.g. through the DNIC loop? Another explanation could be that the afferents from visceral organs are organised differently in the spinal cord. Hence, it is normally believed that they terminate in

lamina I/II and V (Suguira & Tonosaki 1995) and thus is subject both deep and superficial modulation.

6.4 DNIC

One unique inhibitory mechanism is the phenomenon termed DNIC. Some of the neurones in the dorsal horn of the spinal cord are strongly inhibited when a nociceptive tonic stimulus is applied to any part of the body, distinct from their excitatory receptive fields, underlying the term “pain inhibits pain”. DNIC influence only convergent neurones: the other cell types which are found in the dorsal horn, including specific nociceptive neurones in lamina I and II, are not affected by this type of control (Lautenbacher et al. 2002). The inhibitions are extremely potent, affect all the activities of the convergent neurons and persist, sometimes for several minutes, after the removal of the conditioning stimulus. DNIC are sustained by a complex loop which involves supra spinal structures since, they cannot be observed in animals in which the cord has previously been transected at the cervical level.

The ascending and descending limbs of the DNIC-loop travel through the ventro-lateral and dorso-lateral funiculi respectively. DNIC result from the physiological activation of brain structures putatively involved in descending inhibition. However, based on animal data, lesions of the following structures did not modify DNIC: PAG, cuneiform nucleus, parabrachial area, locus coeruleus/subcoeruleus or RVM including raphe magnus, gigantocellularis and paragigantocellularis nuclei. By contrast, lesions of the DRN in the caudal medulla strongly reduced DNIC (Le Bars 2002; Villanueva & Le Bars 1995). Thus it has been proposed that DRN was exclusively inhibitory (Bouhassira et al. 1992). A schematic drawing is shown in figure 8. However other studies have proposed that DRN is also involved in descending facilitation (Lima & Almeida 2002). The classical animal studies examining diffuse noxious inhibitory control show inhibition of spinal dorsal horn neurons following noxious heterotopic stimuli (Dickenson & Le Bars 1987; Millan 2002).

In man the reticular system in the brainstem and probably spinoreticular tracts are also believed to be key neuronal links in the loop sub serving DNIC in man (De Broucker et al. 1990). Neurones within the DRN consist of multiceptive neurons which have the whole body as receptive field and the descending projections involved in DNIC, terminate in the dorsal horn at all levels of the spinal cord (Pud et al.2009).

The involvement of supra-spinal structures are supported by a study, which have showed that psychological parameters can shape the DNIC response, as it has been shown that expectation of hyperalgesia completely blocks the DNIC effect (Goffaux et al. 2007). DNIC reduces the pain perception from a primary stimulus, and can be induced experimentally by heterotopic tonic pain stimuli outside the receptive field of the primary stimulus (Graven-Nielsen et al. 1998; Song et al. 2006; Wilder-Smith et al. 2008). Analogous results have been obtained by means of combined psychophysical measurements and recordings of nociceptive reflexes.

We believe that the hypoalgesia in study II (shown to electrical stimulation of oesophagus and rectosigmoid) after chemical perfusion was a result of descending inhibition. This may likely be caused by DNIC-induction following the chemical perfusion. The study showed a modality-specific activation of central mechanisms. The decreased response to electro-stimulation indicated an activation of descending control but the nociceptive-specific neurones of the superficial lamina (heat and mechanical) were not inhibited. If so, it could be caused by an activation of the wide-dynamic neurones in lamina V. An earlier study, in which DNIC-effect on spinal activity was selective upon different mechanisms, supports these findings (Witting et al. 1998).

Figure 8

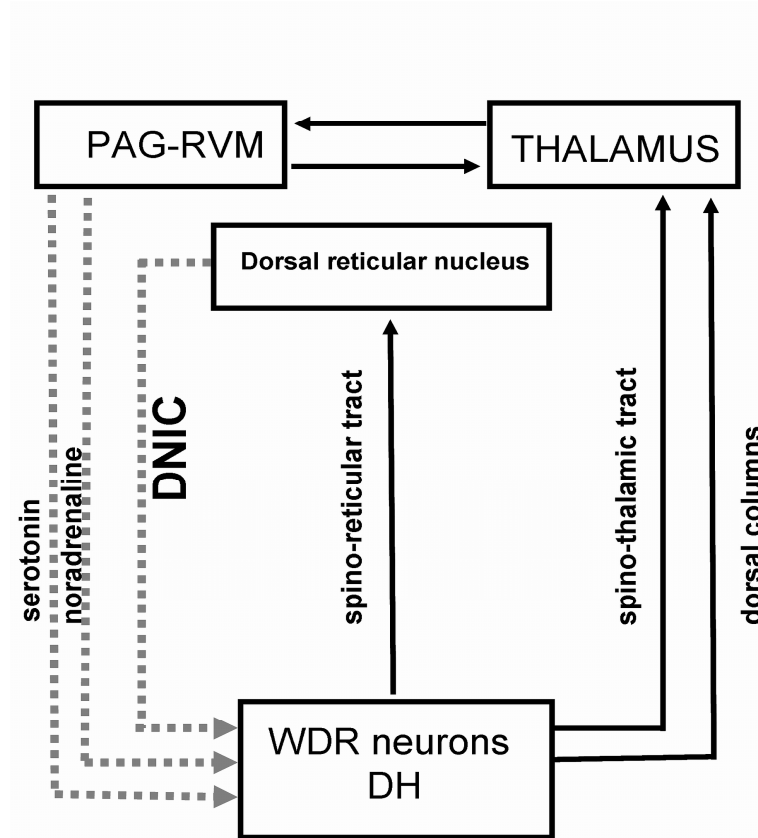


Figure 8: The spinal-reticular-spinal loop, consist the mechanism behind DNIC. However, DNIC is probably not completely separated from other inhibitory circuits. The loop involves, the dorsal reticular nucleus, which is a part of the brainstem-circuit, which can either inhibit or fascilitate pain. It has been shown that psychological parameters can shape DNIC response, e.g. expectation of hyperalgesia can completely block DNIC. Hence, the loop is likely a part of an integrated inhibitory central control which involves both DNIC and modulation through thalamus and the PAG-RVM.

6.5 The brain stem inhibitory circuit

Classical animal studies have shown that electrical stimulation of the PAG resulted in descending inhibition. The PAG do not project directly to the spinal cord. Instead its principle descending projection is to the RVM, which can be considered the output of the midline pain-modulation system. Functionally, RVM is defined as the medullary-pontine area, in which electrical stimulation or opioid injection produces behavioural anti-nociception in animals. RVM includes the reticular formation and projects diffusely to the dorsal horn laminae (Fields & Heinricher 1985). The RVM modulatory system causes either inhibition through “OFF-cells” or facilitation through “ON-cells” on recipient dorsal horn neurones (Fields & Heinricher 1985; Gebhart 2004; Heinricher & Neubert 2004; Millan 2002; Ren & Dubner 2002). Hence the role of RVM nociceptive information is

bidirectional. A shift in the balance between ON- and OFF cell populations such that ON-cells predominate, underlies likely the pro-nociceptive influence which is present in chronic inflammatory and nerve injury states (Porreca et al. 2002). It is worth to notify that RVM is in close vicinity with the dorsal reticular nucleus (involved in the spino-bulbo-spinal loop), and therefore the two descending mechanisms may communicate, as a part of an overall modulating circuit.

6.6 Top-down cortico-thalamo-brainstem inhibitory circuits

There is no absolute anatomical separation between structures involved in the top-down descending facilitation or inhibition and most centres exert more than one modulating effect. Top-down mechanisms are involved in endogenous cognitive and affective processes. The person's expectation and earlier experiences influence directly on the pain perception (Ploghaus et al. 2001; Ploghaus et al. 2003; Tracey et al. 2002). Distraction, which is also used in pain-coping techniques, results in lesser pain, whereas anxiety and fear facilitate the pain. The major brain-sources involved in descending inhibition are limbic structures such as anterior cingulate cortex and networks to hypothalamus, prefrontal cortices, amygdala and brainstem areas such as PAG and RVM, see figure 9.

In study III, we observed strong cingulate activation after DNIC induction, and hence we suggested that the cingulate cortex may play a coordinating role to the frontal-cortico-limbic-brainstem top-down inhibitory network. If so, the findings may reflect a communication between this inhibitory brain circuit and DNIC, possibly through limbic communication with the dorsal reticular nucleus, as it has also been suggested by (Heinricher et al. 2009; Goffaux et al. 2007).

Figure 9

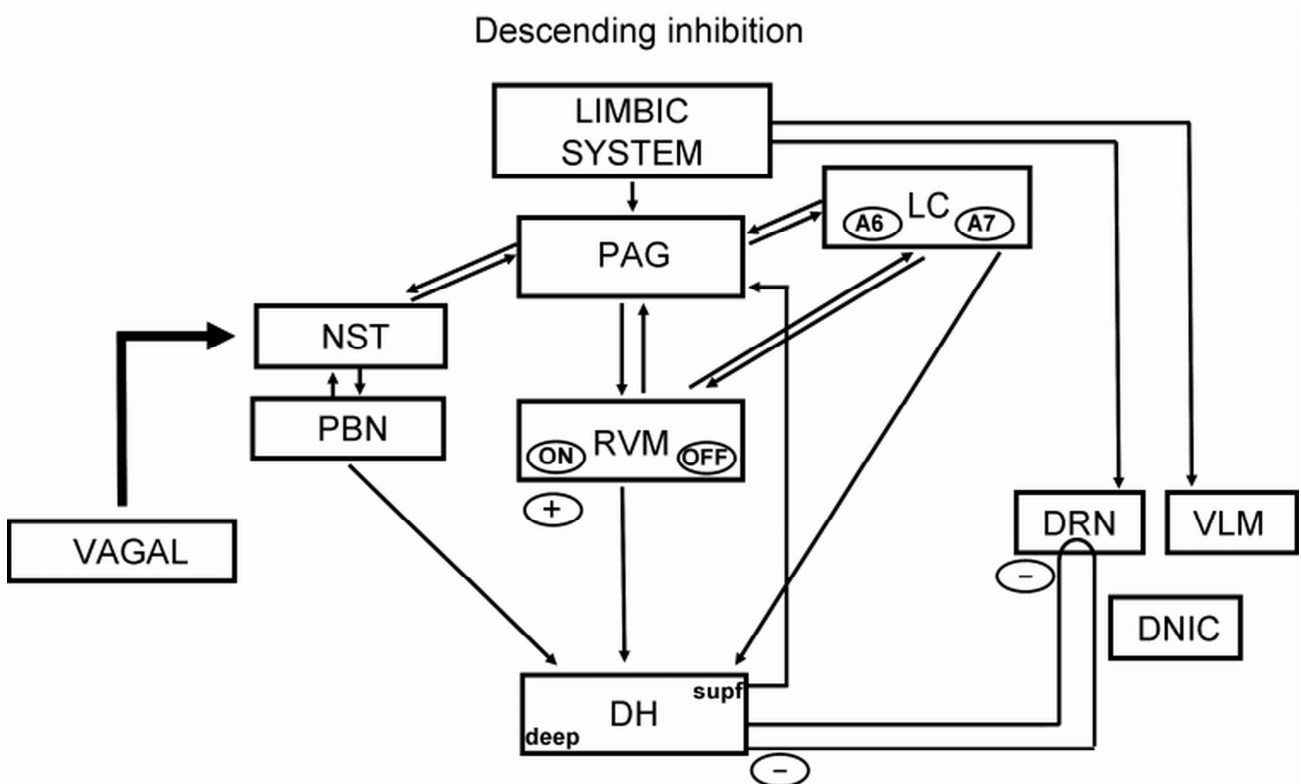


Figure 9: A schematic drawing of the complexity of the neuronal matrix involved in descending pain control. The used abbreviations are: DH: dorsal horn; RVM: rostro-ventral medulla, PAG: periaqueductal grey, LC: locus coeruleus; NST: nucleus of the solitary tract, PBN: parabrachial nucleus; DRN: Dorsal reticular nucleus, VLM: ventro lateral medulla; DNIC: diffuse noxious inhibitory control. Visceral inputs relay in a viscerotopic manner in NTS, which projects viscerotopically to the PBN and vagal activation has been shown to interact directly with the descending control, including inhibition. The spino-bulbo-spinal loop involves the dorsal reticular nucleus is termed DNIC. This descending control is in many cases considered independent of the other central descending mechanisms. However, direct communication from the limbic system to the dorsal reticular nucleus has been proposed, and hence it may not be considered as a completely isolated system.

Modified from (Benarroch 2006; Calvino & Grilo 2006; Goffaux et al 2007; Heinricher et al 2009)

6.7 Descending facilitation

A characteristic of the descending pain modulation arising in the RVM have been described in details (Millan 2002; Fields & Basbaum 1999; Morgan et al.1994). Such descending modulation is believed to travel in the dorsolateral funiculus to the spinal horns (Fields & Basbaum 1999). The dorsal reticular nucleus has – in contrast to earlier findings (Bouhassira et al. 1992) – been proposed as being purely facilitating (Lima & Almeida 2002). The effect on the dorsal horn neurones is primarily on the superficial interneurons in lamina I and II, which possesses the ability of being both inhibitory and excitatory (Maxwell et al. 2007). Hence, descending facilitation activates the

same pathways and neurons as the descending control, and the balance between inhibition and facilitation is dynamic (Heinricher et al. 2009). The importance of descending pain facilitation under physiological conditions is unclear, but could be explained through a limitation of tissue damage (Millan 2002). Besides the serotonergic and adrenergic neurotransmitters (which are involved in both descending facilitation and inhibition) Table 1 provides an overview of the principle pathways which directly modulate the nociception.

Table 1: The principle pathways, which directly modulate the nociception of the dorsal horn neurones. Only the primary transmitter substances and receptor types are listed. Modulated from (Millan 2002)

Anatomical structure	Transmitter substances	Anti-nociceptive	Pro-nociceptive
Hypothalamus	Dynorphin, enkephalin, nitric oxide, GABA, histamine, CGRP and others	Yes	Yes
Parabrachial Nucleus	?	Yes	
N. of the solitary tract	?	Yes	Yes?
RVM	Acetyl Choline, GABA, glycine, enkephalin, cholecystokinin	Yes	Yes
Locus coeruleus	Noradrenaline, GABA, glutamate, enkephaline, galanin	α_{2A} , α_{2B} ,	α_{1A} , α_{2A} ,
Dorsal reticular nucleus	?	Yes	Yes
Anterior cingulate cortex	?	(Yes) study III	Yes
Pre-frontal	?	Yes	
PAG	Cholecystokinin		Yes

6.8 Autonomic influence on descending control

As described earlier in section 3.3 the dorsal horn neurons project to several regions of the medulla, pons, and midbrain via spinobulbar (ie, spinoreticular and spinomesencephalic) pathways, see figure 3. These projections provide nociceptive and viscerosensory input to brainstem neurons that initiate autonomic, endocrine, and antinociceptive responses via descending projections to the spinal cord, ascending projections to the forebrain, or both. The vagus has a sensory nucleus: nucleus of the solitary tract (NTS) and two brainstem motor nuclei (Benarroch 2001). Visceral inputs relay in a viscerotopic manner in NTS, which projects viscerotopically to the parabrachial nucleus (PBN) (Cortelli & Pierangeli 2003). Brainstem autonomic nuclei integrate incoming interoceptive signals

such as pain, with descending modulation, homeostatic and defence motor outputs (Benarroch 2001; Benarroch 2006).

6.9 Disinhibition

Impairment of the human descending inhibitory control; or related facilitatory mechanisms, or both, covers the term *disinhibition*. Disinhibition has been proposed to potentially underlie the pathogenesis in both chronic somatic and visceral pain (Mitchell et al. 2004).

A human experimental study showed that muscle pain impaired descending inhibition the following way: Two concurrent conditioning tonic pain stimuli caused less DNIC compared with either of the conditioning stimuli given alone (Arendt-Nielsen et al. 2008). Hence, the authors conclude that this finding may explain why patients with chronic musculoskeletal pain have impaired DNIC.

Disinhibition underlies likely the pathogenesis in patients with temporomandibular disorders (Bragdon et al. 2002), chronic low back pain (Peters et al. 1992), fibromyalgia (Lautenbacher & Rollman 1997), complex regional pain (Drummond & Finch 2006), painful osteoarthritis (Kosek & Ordeberg 2000) and chronic tension-type headaches (Sandrini et al. 2006). In contrast to musculoskeletal pain little information exists regarding disinhibition in painful gastrointestinal diseases, but some evidence has been shown in patients suffering from chronic pancreatitis and irritable bowel syndrome (Coffin et al. 1994; Drewes et al. 2008; King et al. 2009; Mayer & Gebhart 1994; Wilder-Smith et al. 2004). Recently a study showed that DNIC efficiency predicted lower incidence of developing chronic post-thoracotomy pain (Yarnitsky et al. 2008). The authors foresee a possible pain profile based on tests including DNIC-induction, as part of an effective pain management tailored for each individual.

VII: Experimental pain

7.1 Rationale for experimental pain

One of the rationales for experimental pain models is to control the confounding factors, which often underlie the picture of clinical pain. Hence, assessment of basic GI functions, mechanisms of disease and treatment efficacy, can be standardized. Under these circumstances the investigator controls the experimentally induced pain (including the nature, location, intensity, frequency and duration of the stimulus), and provides quantitative measures of the psychophysical, behavioural or neurophysiologic responses (Andersen et al.2000; Arendt-Nielsen 1997; Drewes et al).

Different experimental animal models have been used in this context. The advantages of these models are obvious: neuronal activity can be studied directly in anesthetized or spinalized animals with invasive recording techniques or via assessment of behaviour (Sengupta & Gebhart 1994). However, as neurobiology of the pain system differs substantially even between animal species, translations from animal studies to human pain studies have some major shortcomings.

7.2 Experimental pain models in humans

Human experimental pain studies have gained much interest during recent years. In man, pain is closely related to culture, linguistic terms and expressions and should be regarded as the net effect of complex multidimensional mechanisms involving most parts of the CNS including intensity coding, affective, behavioural and cognitive components. These factors explain some of the difficulties and challenges in quantifying human sensory experiences with simple neurophysiologic and/or behavioural methods, and why interest in more advanced human experimental pain studies has increased rapidly during the last decade (Curatolo et al.2000; Drewes et al. 2003). The ultimate goal of advanced human experimental pain research is to obtain a better understanding of pain mechanisms involved in pain transduction, transmission, and perception under normal and pathophysiological conditions, such as clinical pain. Obviously the risk of perforation and other complications during invasive procedures limits the testing possibilities within gut stimulation. Due to these difficulties in accessing the GI tract, visceral experimental pain testing is far more resource-intensive and challenging than the more traditional somatic pain-stimulations.

7.3 Multi-modal sensory testing

Ideally, experimental stimuli to elicit gut pain in man should be physiologic, minimally invasive, reliable in test-retest experiments and quantifiable. Preferably the pain should mimic observations in diseased organs by inducing phenomena such as allodynia and hyperalgesia. Previous visceral studies, which have assessed sensory testing of the rectum, have relied on relatively simple mechanical or electrical stimulation. These methods are easy to apply, but they have numerous limitations (Drewes et al. 2003). As pain is a multidimensional perception, the response to a single stimulus of a given modality only represents a limited fraction of the entire pain experience. Hence the development of a multimodal probe to assess rectosigmoid sensation was warranted (study I-III), see figure 10.

Figure 10

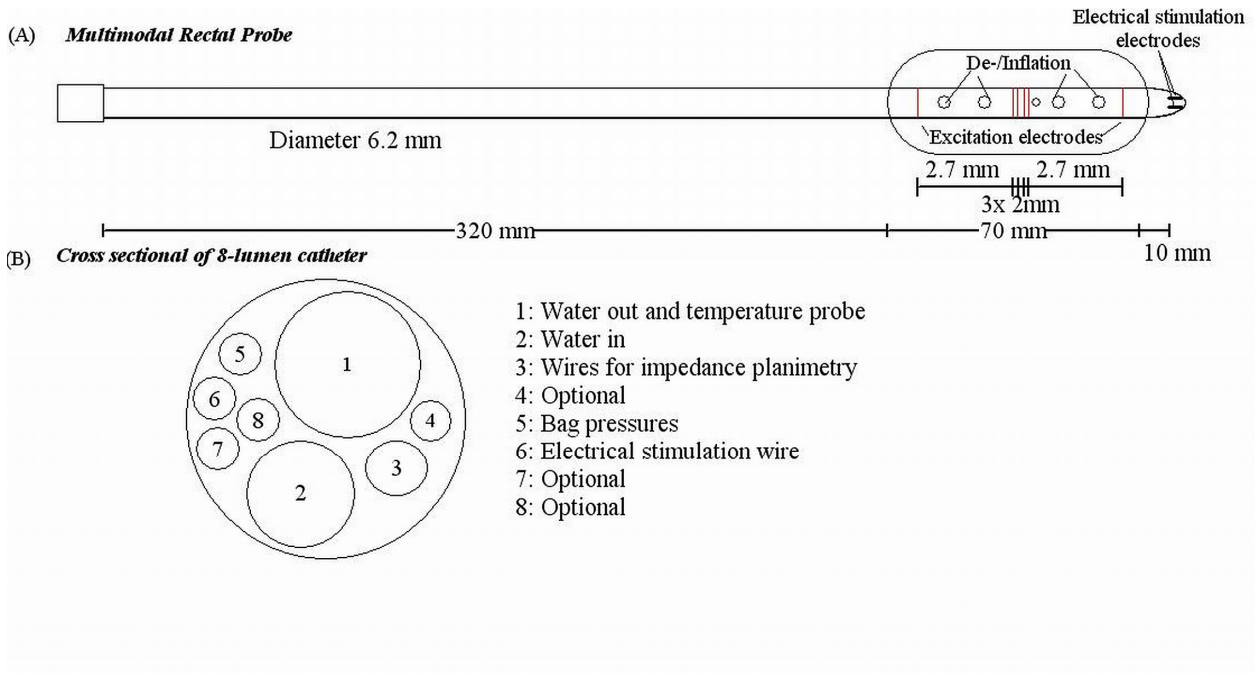


Figure 10: The development of the multimodal rectosigmoid probe was warranted, because it could provide the investigators with receptor and pathway differentiated knowledge. The different stimulus modalities include 1) electrical stimulation of the rectosigmoid junction, 2) thermal stimulation through circulation of hot or cold water through the bag and 3) mechanical distension including two sets of detection electrodes used for impedance planimetry.

7.3.1 Methodology

The development of a multimodal rectal probe made it possible to stimulate electrically (with either single pulses or trains); thermally (with either heat or cold) and mechanically (distension based on

volume and pressure, but also a biomechanic profile obtained through measurement of the cross-sectional area). Reproducibility of the probe is described in details in study I, but it proved to be reproducible across days in all modalities.

The probe is currently integrated in new protocols investigating functional pathophysiology in patients suffering from pain in the following projects:

- 1) Investigation of patients with chronic pancreatitis, Aalborg
- 2) Investigation of patients suffering from IBS, before and after temporary sacral nerve stimulation, Århus
- 3) Investigation of patients suffering from megarectum, London.
- 4) Investigation of pharmacological interventions in healthy volunteers, Aalborg
- 5) Investigation of the pain relief in patients suffering from chronic pancreatitis, before and after treatment with pregabalin, Aalborg.

Future studies include

- 6) Investigation of a multi-segment, multi-tissue study in patients suffering from Diabetes Mellitus, Bergen
- 7) Investigation of faecal evacuatory disorders, London.

7.3.2 Electrical stimulation

Electrical stimulation of the GI tract has been used to study, for example, basic pain mechanisms (Arendt-Nielsen et al. 1997; Drewes et al. 1997; Drewes et al. 1999a; Drewes et al. 2002; Ness & Gebhart 1990) via evoked brain potentials to gut stimuli (Hollerbach 1997; Sami et al. 2006; Sarkar et al. 2000), and the effect of analgesics in both healthy volunteers and patients (Staahl et al. 2006a). The main advantage of electrical stimulation is its reproducibility (Staahl et al. 2006b; study I). Furthermore, its dynamic range (i.e., the range from sensation to pain threshold) is relatively high, allowing more robust assessment of sensory thresholds. A further advantage is that electrodes are easily implemented on different GI-probes. The well defined on- and offset of the stimulus makes it suitable to study pain mechanisms related to time such as temporal summation (Drewes et al. 1997; Drewes et al. 1999b) and cerebral evoked potentials.

There are, however, also limitations and drawbacks. Depending on the probe-design and the electrodes, it may be difficult to obtain optimal mucosal contact between the electrodes and the GI

tract. Impedances between electrodes and mucosa should preferably be less than 3 k Ω before stimulation is initiated.

Electrical stimuli are neither natural nor specific for any sensory modality. Hence, it bypasses peripheral receptors and stimulates all afferent nerves directly, including the silent fibres. However, as most gut afferents are polymodal and respond to a wide range of stimuli, specificity may be of minor importance (Su & Gebhart 1998). In that case electro-stimulation reflects the central nervous response rather than peripheral afferents. However, the action potential of the sensory nerves is partly determined by the trans-membrane electrical potential and partly by the nerve-properties including myelin and ion-channel figuration. Thus, there may after all be some selectivity, relating to fibre type as the non-myelinated afferents (C-fibres) possesses higher activation threshold than myelinated A-fibres (Curatolo et al. 2000; Handwerker & Kobal 1993; Tougas et al. 1993).

Numerous stimulation paradigms have been recommended but no general consensus exists with respect to the configuration of the optimal electrical pulse. In fact the stimulus should reflect the purpose, e.g. is it crucial to use single pulses in electrophysiological studies, where early peaks of evoked brain potentials are wanted. On the other hand a single stimulus in the gut demands rather high intensity to evoke pain, and trains or continuous series of pulses (study I) can be used in order to investigate temporal summation to a repeated series of stimuli (termed “wind-up” in animal experiments).

As gut-segments exhibit differences in anatomy and innervations, a general consensus regarding stimulation location is warranted. Thus future studies should include standardized and validated optimal parameters such as stimulus duration, pulse-shape, polarity, frequency and intensity allowing comparison between different laboratories.

In study I, which was based on psychophysical assessments, we stimulated with a “single” pulse consisting of 5 monophasic square pulses, each having duration of 1 ms, delivered at 200 Hz. The repeated stimulus used in this study, consisted of 5 “single” pulses applied at 2 Hz.

In study II and III we improved the electrical stimulation to a single stimulus consisting of one monophasic square pulse of 2 ms duration. This improvement was important to minimize the length of the stimulus artefact in the recorded evoked potentials.

7.3.3 Electrical field

Electrical stimulation creates an electrical field in the surrounding tissue. Hence a stronger field is needed to stimulate the non-myelinated C-fibres.

In order to visualize the electric field, we developed a model in the oesophagus based on stimulation with either ring or patch electrodes. The electrical field in the different oesophageal layers was computed using a finite element model based on a 3D model (mucosa, muscle layers and surrounding tissue). Each layer was assigned different electrical properties. An electrical field in excess of 20 V/m was considered to activate the afferents originating from the esophagus. The threshold of 20V/m corresponds with the threshold for action potential in a non-specific nerve (Holdefer et al.2006).

FEM provides analysis of the electric field in a defined volume conductor. The geometry of the oesophagus was determined in an earlier experiment using endoscopic ultrasound images (Frokjaer et al. 2006). The thicknesses of the mucosa and muscle layers were 0.85 mm and 2.72 mm respectively, resulting in a total wall thickness of 3.57 mm. In the current model the conductivity for the mucosa layer was set to 0.05 S/m (IFAC 2008) and for the muscle layer to 0.53 S/m (IFAC 2008). The volume of the surrounding tissue-layer was considered infinite with a conductivity of 0.27 S/m (IFAC 2008).

Figure 11 shows the visualization of the electric field.

Figure 11:

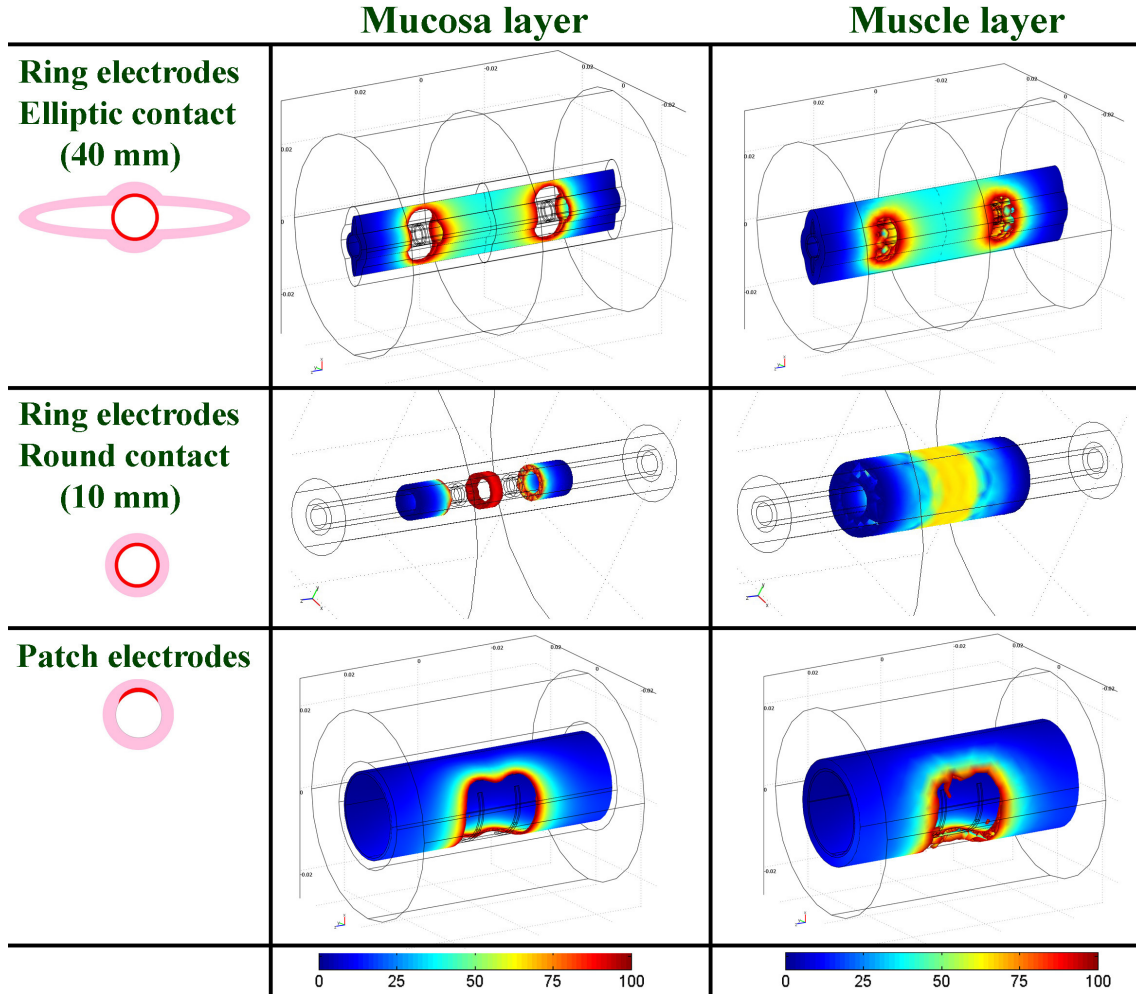


Figure 11: Color coded graphs of the voltage distribution for the mucosa and the muscle layers computed using FEM. The range of colors from blue to red represents the range from 0 to 100 V/m. The stimulation current I was in all cases 20mA. The first column illustrates the geometry of different contact between electrodes and mucosa. The upper row of graphs is obtained from ring electrodes and an elliptical contact area with the mucosa. The middle graphs are obtained from ring electrodes with a circular contact area to the esophageal mucosa. The bottom graphs are obtained from patch electrodes in contact with the mucosa.

By use of the sensory scores from 2 subjects (sensory threshold and pain detection threshold) as input to the FEM, new information was provided regarding the electric field and involvement of the different anatomically layers of the oesophagus, see figure 12. However, because the nerve density in the different oesophageal layers is unknown, we can only predict an association between the electrical field and the subjective pain score. Hence, the model needs more development before it can be validated in a larger number of healthy volunteers.

Figure 12

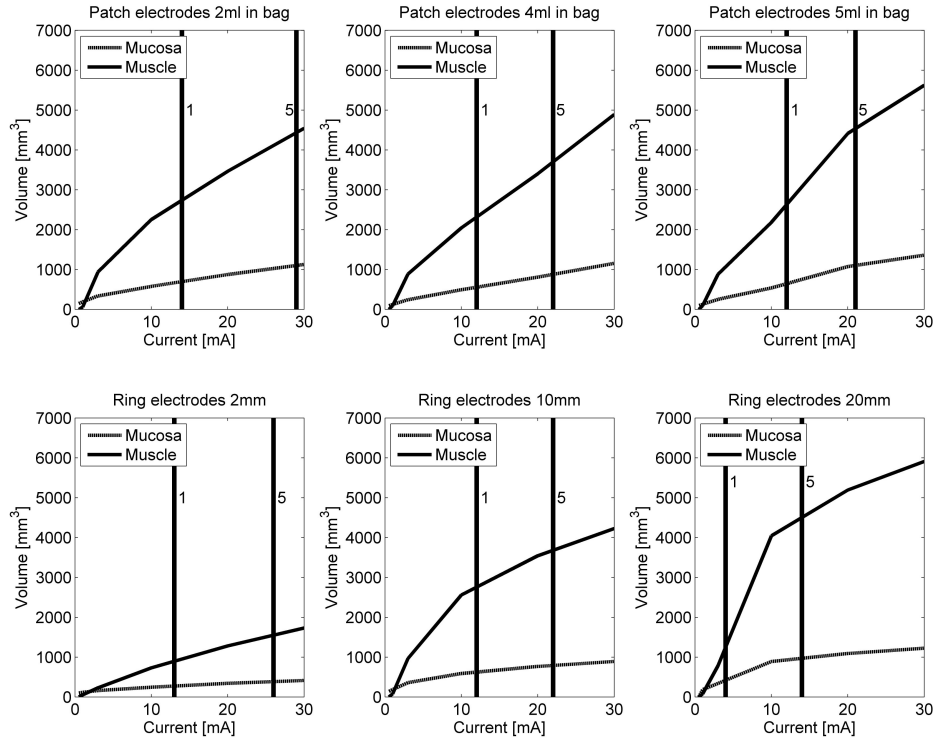


Figure 12: Illustration of the electrical field (expressed as a volume) which exceeds a threshold of 20 V/m in the three anatomical layers. The stimulation current range of the different stimulation electrodes is illustrated as the window between the sensory threshold (1) and the pain detection threshold (5). The grey line represents the mucosa involvement and the black line represents the muscle involvement. The upper row represents patch electrode mounted on a bag with different filling: 2, 4 and 5 ml. The lower row represents ring electrodes placed with an inter-electrode distance of 2, 10 and 20 mm

7.3.4 Thermal stimulation

In contrast to mechanical and electrical stimulation, thermal stimuli activate receptors selectively. The mucosal heat-responsive TRPV1 receptors and mucosal cold-responsive TRPA1 are activated with temperatures above 43° and less than 17° respectively (Chan et al. 2003). Thermal stimulation has been used to study basic pain mechanisms; functional and organic gut disorders and analgesic efficacy in both healthy volunteers and patients (Staahl et al. 2006a). Rectal heat pain stimulation has been performed using a peltier device (Chan et al. 2003). In our laboratory cold or heat stimulation is based on recirculation of cooled and/or heated water in the bag with a temperature-sensor placed inside the bag, see figure 13. In study I, sixty degrees of hot water was circulated in the bag, which produced a maximum stimulation temperature of 52°. In study II we wanted to

assess within the painful range (moderate pain) and therefore we raised the water temperature to 68°. Cold stimulation was done in study I by circulating 4°. To analyze data, the most reliable proxy of the thermal energy applied is the area under the temperature curve (Pedersen et al. 2004).

Figure 13

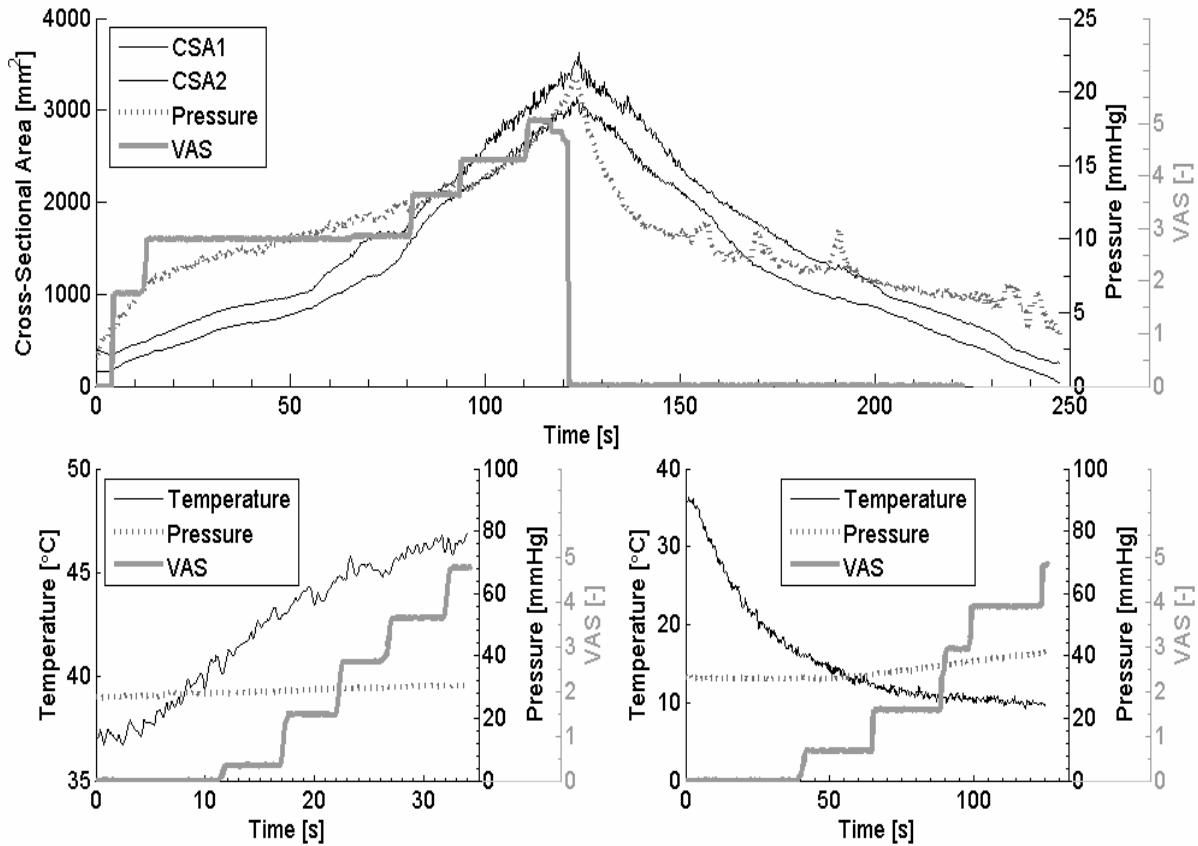


Figure 13: The top: On-line data acquisition including two-channel impedance planimetry measurement in the rectum. Distension was done until pain detection threshold was reached or after 120 seconds, corresponding to 240 ml (bag capacity). At this point, the deflation of the bag was done with same perfusion rate as the inflation. The CSA curves show a non-linear pattern, which is similar to the sensory score on the visual analog scale (VAS). The sensory score remains on a plateau while CSA does not increase due to the filling of the bag, whereas the pressure assessment within same period increases. In this period bag distension induces relaxation distally as a result of an inhibitory reflex, hence distributing the volume distally. When the bag is filled in a more radial direction, the CSA and the sensory score is increased. The bottom: On line data acquisition demonstrating the thermal stimulation with heat and cold.

7.3.5 Mechanical stimulation

Methods based on impedance planimetry allows recordings of luminal cross-sectional area directly and calculation of the radius in the distended GI-segment (Drewes et al. 2002; Drewes et al. 2003a; Gao et al. 2002; Gregersen et al.1999; Gregersen et al.1988; Petersen et al. 2003). Estimates of circumferential wall tension, stretch and strain based on measured radius are more accurate than estimates based on volume exclusively (Gregersen & Kassab 1996), see figure 13. However, the method has some limitations. First the method assumes an acceptable circularity of the investigated gut segment. Due to the complex anatomy and the asymmetrical filling of the rectum we believed that several CSA measurements would give a more reliable profile of the distension-evoked changes in shape. However, tone of the rectal wall and transversal folds also influences the CSA measurements. Consequently, we decided to use two pairs of electrodes, using the average value for further analysis.

During rectal distension in study I, stretch ratio at pain detection threshold produced an excellent intra class coefficient of 0.98, both with and without administration of the antimuscarinic drug butylscopolamine. It is our experience that CSA only is reliable to use if the CSA does not exceed 6500 mm². In order to reliably compute, e.g., rectal stress and strain during distension, more complex modelling such as multiple CSA recording would be an option in future experiments.

In study II, we changed to a larger bag size in order to reach moderate pain level. As CSA measurements were not reliable during these rather big distensions, the mechanical calculation in study II were based on volume.

7.3.6 Chemical perfusion

In order to resemble clinical inflammation of the GI tract and approach the ideal experimental visceral pain stimulus, chemical stimulation has been widely used (Ness & Gebhart 1990). Such stimuli have successfully been applied to the skin and muscles, but are also widely used in the gut (Andersen et al.1995; Arendt-Nielsen 1997; Curatolo et al. 2000; Babenko et al. 1999).

Oesophageal acidification is commonly used to sensitize the gut evoking allodynia/hyperalgesia, but the model may also be used for direct stimulation (Bernstein & Baker 1958; Fass et al. 1998; Tack JF 1999). The major relevance of the model may be induction of sensitization of visceral afferents to subsequent experimental stimulation. Chemical stimulation has been used to study, for example, basic pain mechanisms and functional gut disorders (Study II, Drewes et al.1997; Drewes et al.1999a; Drewes et al. 2002; Drewes et al. 2006a; Frokjaer et al.

2005a; Hobson et al. 2004; Matthews et al. 2008; Sarkar et al. 2000). However, drawbacks of chemical stimuli may include a relative long latency time to onset of effects, and often the effects can not be reproduced (Ness & Gebhart 1990).

Capsaicin exhibits a *local* activation of TRPV1 channels, and animal studies have documented a synergistic effect between acid and capsaicin, leading to hyperalgesia (Caterina et al. 1997; Tominaga & Tominaga 2005). Conversely, in-vitro studies with animal tissues have shown that capsaicin desensitizes the TRPV1 receptor through dephosphorylation by calcium-induced calcineurin activation and extensive capsaicin-induced TRPV1 receptor stimulation in-vivo may lead to desensitization partly as a result of local substance P depletion (Docherty et al. 1996). Capsaicin has been used to evoke pain in the small and large intestine (Drewes et al. 2003b; Hammer & Vogelsang 2007; Lee et al. 2004). Moreover, capsaicin has been used to explore basic functions such as autonomic changes in the referred pain area (Arendt-Nielsen et al. 2008b).

Based on the animal-findings showing synergistic effect, and as an attempt to improve the classical acidic perfusion, we recently developed a chemical perfusion, which is a combination of acid and capsaicin. The method was proved in the human oesophagus, in which all 15 subjects were sensitized (Olesen et al. 2009). The perfusion induced locally reproducible hyperalgesia to subsequent heat and electrical stimulation and an expansion of referred pain areas. The increased referred pain areas reflect convergence mechanisms on second order neurons in the spinal cord and can be used to elucidate the central component of the hyperalgesia (Olesen et al. 2009).

In study II, we integrated the improved chemical perfusion to the multi-segmental study, which explored the basic central pain mechanisms following oesophageal perfusion. Electrical stimulation of the oesophagus and a multimodal approach to rectosigmoid were included. We showed rectal hyperalgesia to mechanical and heat stimulation following oesophageal perfusion, and hence we concluded that the perfusion induced central sensitization and viscerovisceral hyperalgesia.

Simultaneously, we observed hypoalgesia to electrical stimulation, which was shown in the oesophagus proximal to the perfusion site and in the rectosigmoid. Due to the relative short time period between perfusion and pain assessment we find it unlikely that the observed hypoalgesia should be caused by peripheral substance P depletion (Lembeck & Donnerer 1981). Hence the combined chemical perfusion activated modality specific central mechanisms involving both central sensitization and activation of the descending control.

7.3.7 DNIC induction

The literature is not consistent on whether there is a relationship between the intensity of a tonic heterotopic stimulus and the magnitude of DNIC- induction with some studies showing that the greater the pain intensity the greater the DNIC (Villanueva & Le Bars 1995); while others do not show this relationship (Baad-Hansen et al. 2005; Pud et al. 2005). The effects of DNIC are known to differ, depending on the magnitude and nature of the conditioning stimulation and stimulated nerve fibres (Dickenson et al. 1980; LeBars et al. 1979a; Millan 2002; Terkelsen et al. 2001)

Experimentally induced DNIC includes various techniques: Tonic heat applied through a thermode on the thigh (Kosek & Hansson 1997; Pielsticker et al. 2005), or by immersing hand in 45°- 47° C water (Granot et al. 2008); tonic cold pain (cold-pressor test) (Edwards et al. 2003; Granot et al. 2008; Pud et al. 2005; Lariviere et al. 2007; Potvin et al. 2008; Tousignant-Laflamme et al. 2008; Washington, Gibson, & Helme 2000; Watanabe et al. 1996; Wilder-Smith et al. 2004); tonic ischemic pain by a tourniquet applied to arms or legs (Kosek & Hansson 1997; Kosek & Ordeberg 2000) or tonic muscle pain induced by pressure or injection of hypertonic saline in the trapezius muscle (Ge et al. 2004) or injection of hypertonic saline into the tibial muscle (Graven-Nielsen et al. 1998).

In study III we used the cold-pressor test, which is reliable, cheap and easy to work with (Mitchell et al. 2004). An overview of different techniques used in the cold-pressor test is provided in Table 2. The outcomes of the cold-pressor test are highly dependent of several factors such as accuracy of water-bath temperature, whether the water is circulated during stimulation and the length of the cold-pressor test. No difference in pain-relief between immersing a foot or a hand into the cold-water was found by (Wilder-Smith et al. 2008), whereas the hand was found superior to induce greater pain reduction compared to the foot in (Watanabe et al. 1996). Based on these findings, we used the hand, as it was more pleasant for the volunteer and easier in the experimental set-up. Our hypothesis was, that we could measure alterations in visceral pain assessment and in psychophysical and neurophysiological parameters after a cold-pressor test, in which the volunteers were encouraged to maintain their hand in a circulated cold-water bath ($2 \pm 0.3^{\circ}\text{C}$) for up to 180 seconds. Hence, in study III, we established a model, in which we could investigate the effect of the DNIC- induction on electrical stimulation in the rectum and on the brain-processing of the pain-specific ascending pathway. The psychophysical pain relief was 32%. Neurophysiological assessments showed increase of the latency in the endogenous component P2. This suggests parallel endogenous processing in the inhibitory brain network (Valeriani et al. 2007). Moreover a 39% and 67% increase

of the N1-P1 and P1-N2 amplitudes were shown, (also reflected in the dipole strength) which may indicate a rebound effect.

Table 2: Different techniques for DNIC-induction in healthy volunteers by use of cold-pressor test.

Test pain, Healthy volunteers	Upper Lower Visceral	VAS Thr Neu	All	Men	Women	n	Cold-pressor test Bodypart/°C/sec	Circu- lated
Heat:								
(Granot et al. 2008)				- 46	-16	31	Hand/12;15;18/60	+
(Tousignant-Laflamme et al. 2008)	U	Vas	-37			83	Low arm/7;10;12/120	+
(Edwards et al. 2003)	U	Vas	-10			45	Hand/5;22/60	+
(Washington et al. 2000)	U	Thr	+33			15	Hand/0-5/180	ns
(Watanabe et al. 1996)	U	Neu				16	Hand/0/60-120	ns
(Potvin et al. 2008)	U	Vas	- 29			29	Arm, 7-12/120	ns
(Lariviere et al. 2007)	L	Vas	-18			20	Hand/7/360.	ns
(Lariviere et al.2007)	L	Thr	+ 5			20	Hand/7/360	ns
Electrical:								
(Washington et al. 2000)	U	Thr	+100			15	Hand/0-5/180	ns
(Sandrini et al. 2006)	L	Neu	20				Hand//5-6/300	+
(Wilder-Smith et al. 2008)	Vi	Vas	-45			15	Hand/4/?	ns
(Wilder-Smith et al. 2008)	Vi	Vas	-46			15	Foot/4/?	ns
Study III	Vi	Vas	-32			15	Hand/2/180	+
Mechanical :								
(Pud et al. 2005)	U	Vas		-21	-13	40	Fingers/1/30	ns
(Goodin et al. 2009)	U	Thr		+23	+15	35	Hand/4/180	+
(Arendt-Nielsen et al. 2008)	L	Thr		+64	+30	20	Hand/1-2/300	+
(Wilder-Smith et al. 2008)	Vi	Vas	-36			15	Hand/4/?	ns
(Wilder-Smith et al. 2008)	Vi	Vas	-45			15	Foot/4/?	ns
(Witting et al. 1998)	L	Vas		-25		11	Foot/1/?	ns
Chemical								
(Witting et al.1998)	L	Vas		-18		11	Foot/1/?	ns

The table shows some of the various techniques to induce DNIC experimentally by the cold-pressor test. Description of where the stimulus was applied (upper, lower body or in the viscera). Different techniques have been used to measure the efficacy, hence VAS refers to psychophysical assessment, and the sensory response will be less than baseline; Thr refers to pain threshold, and during DNIC induction this will be higher than baseline; Neu refers to neurophysiological assessment; circulated refers to whether the waterbath was circulated doing the experiment, ns refers to: not stated.

VIII: Electroencephalogram

While traditional metabolic neuro-imaging tools such as f-MRI and PET brain imaging techniques have excellent spatial resolution, their time resolution is relatively poor. Thus, in order to address the sequential order of pain-specific active brain-sources, a method such as EEG which measure neuronal activity directly in real-time, is needed. The electrophysiological tool is widely used to investigate the brain-activity in both health and disease. It is non-invasive and completely harmless. One goal is to use the EEG as a separation-technique which divides the brain response into pain-specific (exogenous) activity and the secondary non-pain-specific (endogenous) activity. Hence the brain-centres involved in the ascending upstream activation can be mapped. The temporal window of exogenous pain processing occurs ≤ 150 ms and endogenous pain processing is ≥ 150 ms post-stimulus (Hobson et al. 2005). Combined with advanced mathematical analysis for dipolar source reconstruction, the use of evoked potentials has improved in order to estimate location of brain activity in real-time on a millisecond-to-millisecond scale.

8.1 Recording techniques of evoked brain potentials

EEG recordings can be used to record cortical evoked potentials (CEP), which is an electrical response in the brainstem or cerebral cortex following a stimulus, i.e. a painful stimulation in the gut. CEP amplitudes are typically lower than the amplitudes of spontaneous EEG (less than a microvolt to several microvolts compared to tens of microvolts for EEG).

Since the CEPs are time-locked to the stimulus and the background activity occurs randomly, the CEP amplitudes become higher during the averaging process, and most of the background noise cancels out. Each peak in the CEP represents a synaptic event associated with the synchronous transmission of afferent information from one group of neurons to another. Recordings of evoked brain potentials have proven to be highly reproducible in studies exploring the afferent processing of visceral sensation in both healthy controls and patients suffering from different organic and functional diseases of the gut (Drewes et al. 2006b; Hobson & Hillebrand 2006; Rossel et al. 2001; Dimcevski et al. 2007).

It has been argued that components of the vertex potentials exhibit highly inter- and intra-individual variation, but never the less they have earlier been used to assess central sensitization or inhibition (Dimcevski et al. 2007; Motohashi & Umino 2001; Oono et al. 2008). Several studies have examined the amplitudes and latencies of painful CEPs in the gut and compared the results between

a control group and a study group (i.e. patients suffering from chronic pancreatitis, non-cardiac chest pain or patients treated with analgesics) (Dimcevski et al. 2007; Hobson & Hillebrand 2006; Hollerbach et al. 2000; Rossel et al. 2001; Sami et al. 2006; Watanabe et al. 2007). Dimcevski et al. showed decreased early CEP latencies in patients with CP compared to healthy controls. Sami et al. showed decreased latencies in the first two positive peaks (P1 and P2) of CEPs following painful stimulation in oesophagus after acid perfusion (Sami et al. 2006). Rossel et al. found shorter latency (P1) and smaller amplitude in patients with IBS compared to healthy controls. Furthermore the control group had a mid-latency positive component after 100ms, which was absent in the patient group and the healthy controls had a single late positive component ($> 150\text{ms}$) whereas the IBS group had a late component which was biphasic (Rossel et al. 2001). The results reflect alterations in the neuraxis during pain processing.

We recorded evoked potentials in both study II and III, by use of a single monophasic square-pulse of 2 milliseconds duration. This short pulse minimizes the stimulus-artefact, and is superior to e.g. stimulation trains, especially in the cases where analysis of the exogenous pain is wanted. In study II we used a relatively simple four-channel system (Nuamp), consisting of two recording electrodes at Cz and Fpz. Reference was placed at the right earlobe.

In study III we used a multiple channel recording system (SynAmp). A display of one of the 64 channel butterfly plot can be seen in figure 14. Reference was placed at the midline, with a Cz close location.

Figure 14

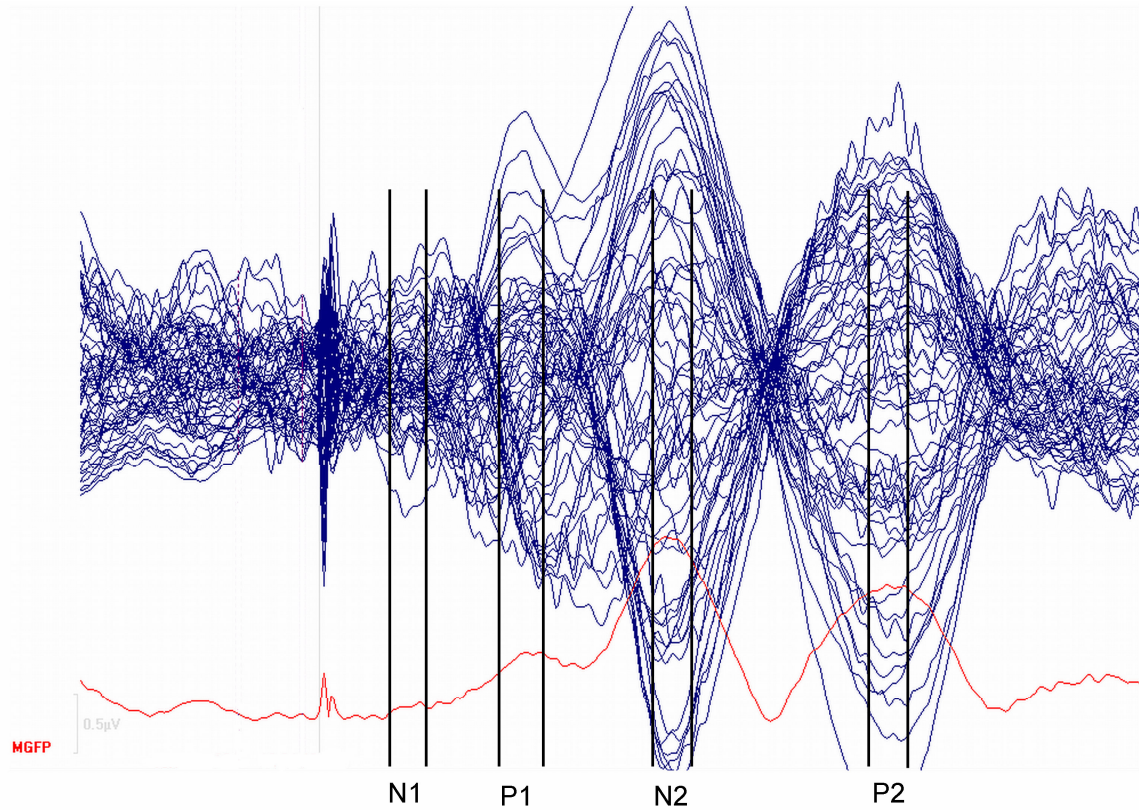


Figure 14: A butterfly plot of evoked potentials to painful rectosigmoid stimulation. The plot shows an acceptable signal to noise ratio. The bottom displays mean global field power. The global field power is independent of the user-selected electrodes. In this case, the mean global field power of the first peak is not as prominent as it is on some of the individual channels.

On the contrary to somatic stimulations, where a substantial number of sweeps (400-800) can be done, we are limited to a relatively small number of stimulations in the viscera. As both recording techniques and post-processing influences directly on the quality of the recorded data, it is important to optimize and standardize each step of the recording- and processing techniques. Hence, we have developed a stimulation paradigm, in which we stimulate with 30 sweeps of a stimulus intensity corresponding to PDT applied at 0.2 Hz. During recordings the room was dimmed, all mobile phones switched off and electrical background noise from e.g. computer screens was avoided. To minimize artefact the subjects lays half-supine with no muscular tension in the neck-region. They were instructed to focus on a spot on the wall, and blink or swallow between stimulations. We recorded with “open filters”, (between 1 and 300 Hz) and a sample rate of 1 kHz.

Each continuous recording-file was analyzed offline (Neuroscan software v 4.3.1, Neuroscan, El Paso, TX, USA) and stimulations containing artefacts were rejected manually. The mean of the accepted stimulations were computed. The procedure consisted of the following pre-processing steps:

- 1) Zero-phase notch filtering (49-51 Hz) with a filter order of 24;
- 2) Zero-phase bandpass filtering (1-70 Hz) with a filter order of 12;
- 3) Epoching in the time window -50 to 350 ms post-stimulus;
- 4) Baseline correction;
- 5) Linear detrending;
- 6) Rejecting sweeps manually and
- 7) Calculating average of accepted sweeps.

In study II and III, latencies and amplitudes of each EP component (N1, P1, N2 and P2) were analyzed at Cz and Cpz, and reproducibility was assessed by comparing the two baseline recordings, see figure 15.

Figure 15

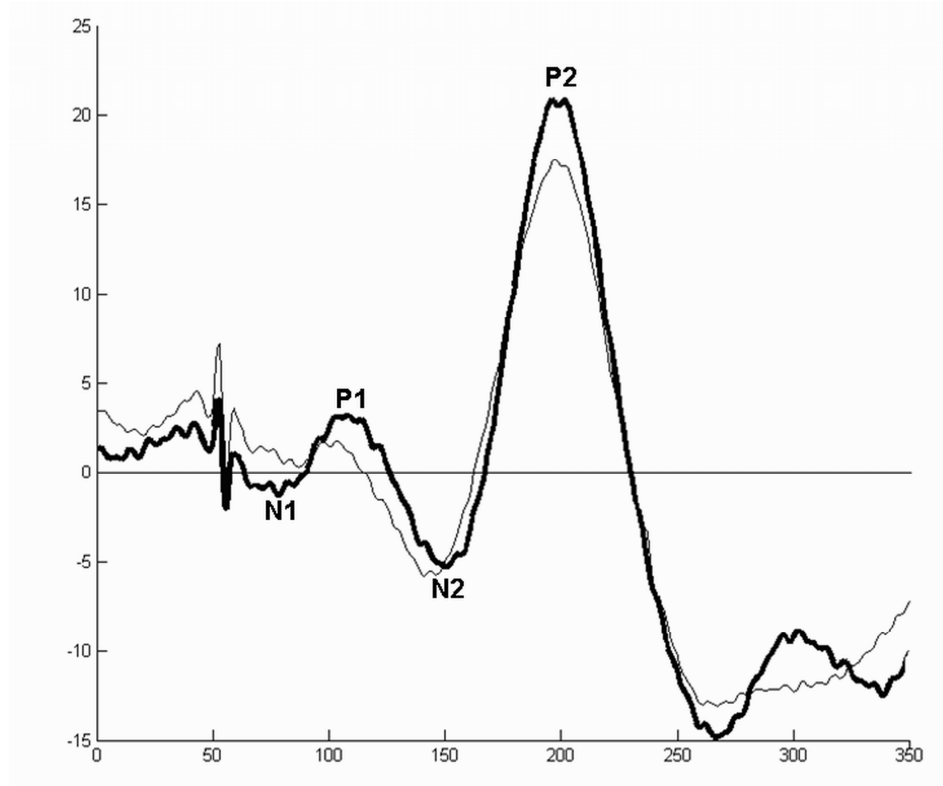


Figure 15: Reproducibility of evoked potentials elicited from 30 painful rectosigmoid stimulations applied at 0.2 Hz. The different peaks N1, P1, N2 and P2 were analyzed in terms of latency and amplitude.

Study II was designed to analyze latencies and amplitudes from painful stimulations elicited in the oesophagus and rectosigmoid after perfusion with randomized placebo or capsaicin+acid. An obvious limitation of this method is the few recording channels, situated close to each other in the midline. We chose the earlobe as reference electrode, in order to obtain large amplitudes. The grand mean of the recorded evoked potentials from the rectosigmoid in figure 16, which shows differences in latency and amplitude between the two perfusion types.

Figure 16:

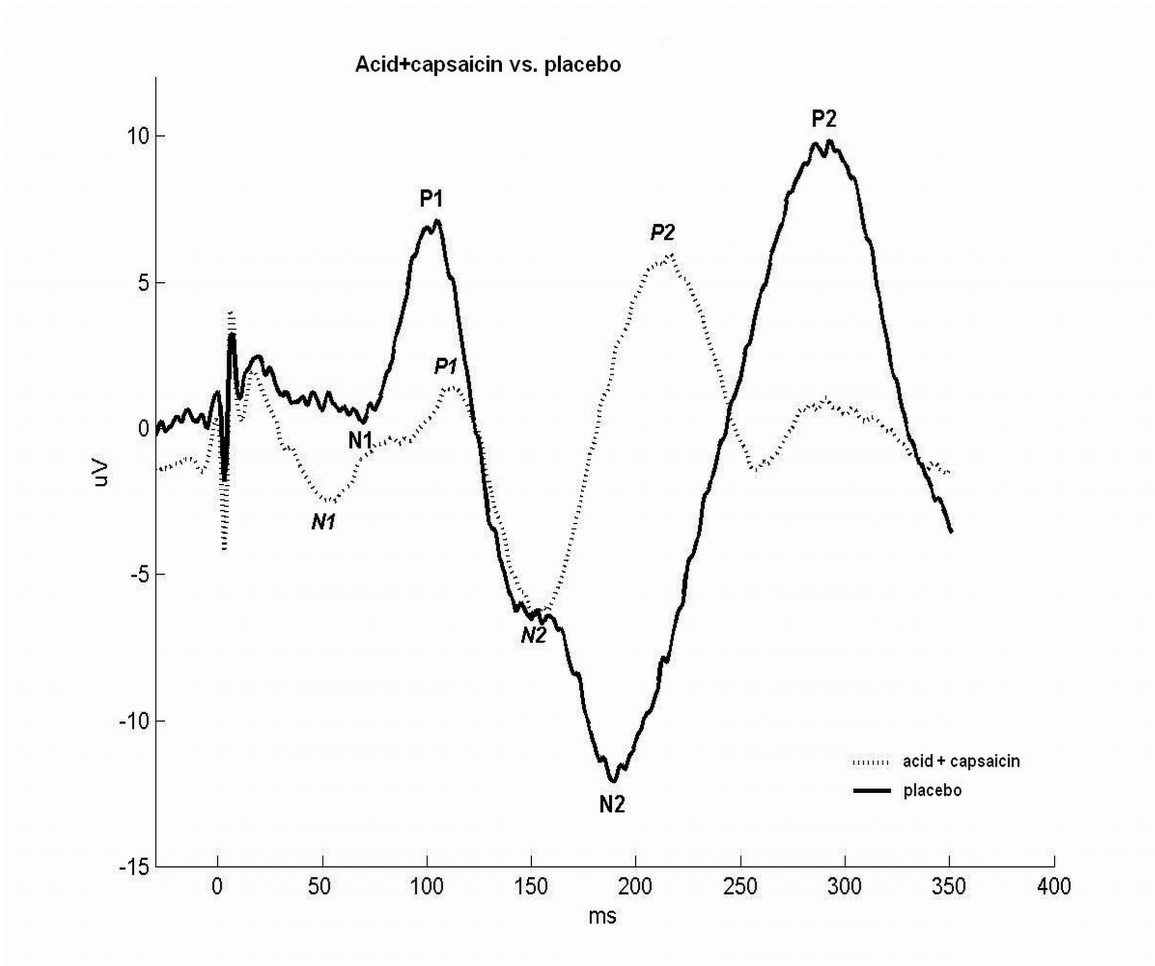


Figure 16: Example of recorded evoked brain potentials to 30 identical rectosigmoid electrical stimulations. The acid+capsaicin perfusion induced a decrease in the N1 and P2-latency. The more rapid onset of the N1 peak (first pain) following the acid+capsaicin perfusion may illustrate an activation of latent collateral pathways. The same phenomenon is present in the late P2-component which to a high degree is influenced by endogenous, cognitive and affective processing. Simultaneously the decrease in all amplitudes may on the contrary be an expression of inhibitory control.

Study II showed reproducible baselines in both oesophagus and rectosigmoid. In comparison to the saline perfusion, a decrease in both latency and amplitude after acid+capsaicin perfusion was seen to rectosigmoid stimulations. The demonstrated changes in latencies and amplitudes most likely explain neuronal changes, such as hyper-excitability (possibly counterbalanced by descending inhibition) in the central nervous system.

The results from study III also showed reproducible baselines. Neither latencies nor amplitudes were affected immediately after DNIC-induction. However, ten minutes after DNIC induction an increase of the latency in the P2 component was present suggesting parallel endogenous processing in the inhibitory brain network (Valeriani et al. 2007). Moreover a 39% and 67% increase of the N1-P1 and P1-N2 amplitudes was shown, (which was also reflected in the dipole strength) may indicate a rebound effect, in which more neurons are activated. The lack of correlation between psychophysics and electrically evoked potentials have been shown in numerous earlier methodological studies (Bromm & Scharein 1982 Buchsbaum et al. 1983; Chapman et al. 1981), and hence the higher amplitudes found in this study, must be interpreted with caution.

8.2 Inverse modelling of cortical evoked potentials

EEG is a mixture of signals deriving from different brain regions arising from synchronous firings of millions of neurons. The EEG signals are not produced by a single source in a well defined location due to the currents flowing to the surrounding tissue via volume conduction. Thus, by the time the signal arrives to the scalp electrodes it is distorted. Hence, it is impossible to predict which electrical brain sources are generating these surface potentials from EEG alone. However, there are mathematical algorithms to estimate source locations based on the scalp potentials. This is known as “inverse modelling”.

The requirements for the recordings are many, before source analysis tools can be applied. Among the critical issues are:

- 1) Number of electrodes: Michel et al. have described the number of needed electrodes to give accurate source estimates (Michel et al. 2004). The work describes a minimum need of a 60-channel system. However the authors recommend the use of a larger number of electrodes, provided that noise (which increases with increased number of electrodes as a consequence of the ill-conditioned character of the inverse problem) is adequately accounted for.
- 2) Positioning of electrodes: Incorrect assumptions of the electrode-positions leads to inaccurate source localization. Hence, individual head shapes influence the estimates. One study evaluated dipole localization error induced by electrode misplacements, and concludes that the localization error was small and nearly negligible compared to error induced by noise (Wang & Gotman 2001). However, the study was only based on 29 electrodes, and thus the spatial distribution for inverse modelling was poor anyway. Even though we mount 64 or 128 channels we always pay

attention to the mounting procedure (distance to nose, symmetric distribution and tight fitting) as well as bringing down impedance in each contributing electrode.

- 3) Position of the reference electrode has direct influence on the waveform analysis and therefore on the amplitude assigned to a certain contour of interest. However, it is irrelevant for source localization as long as the reference is correctly included in the model, because it does not change in any way the biophysical information contained in the potential distribution (Michel et al. 2004).
- 4) Determination of relevant time windows for source estimation e.g. the traditional way to look at the window is “the component-way”. Here latencies of different components assign the time window of interest. In a multiple channel system, this may not be appropriate way of defining the time of interest. Due to different electrode locations on the scalp, there is some latency jitter between electrodes, and therefore we choose our relevant time-window based on the 64 channel peaks in the butterfly-plots.
- 5) Choice of head model has direct influence on the accuracy of the method, and in Curry 6.0 we have the choice between the following options: A spherical head model has homogeneous conductance and its surface represents the scalp. An improvement of this is a head model consisting of 1, 2, 3 or 4 spheres, (corresponding to the brain, cerebrospinal fluid, skull and scalp) each assigned a unique conductance. A more realistic head model is the boundary element method (BEM), which is based on thousands of triangles (Fuchs et al. 2002). The BEM model assumes that multiple compartments have homogenous conductivity. The final solution is the individual head model based on exact digitized electrode position (e.g. polhemus) combined with individual MR-scans.
- 6) EEG noise (electrical or biological) is important to minimize, as it no matter what algorithms is used will influence on the source estimates.

Inverse modelling is based on the idea that groups of neurons, which generate the electrical potential at the scalp, can be modelled by equivalent current dipoles. The inverse problem can only be solved by introducing several a priori assumptions on the generation of the EEG signal. The more appropriate these assumptions are the more trustable are the source estimations. Hence, the assumptions determine whether the solution is limited to only explain the recorded data or if the solution provides neurophysiological information regarding the origin (which brain centres were

activated) of the generated signals. Some of the different solutions to solve the inverse problem are listed in table 3.

Current density techniques such as *Low Resolution Brain Electromagnetic Tomography* (LORETA) display where most of the current is concentrated, and therefore it displays brain areas comparable to imaging tools like PET and f-MRI. On the contrary dipolar source analysis such as *Brain Electrical Source Analysis* (BESA), *Multiple Signal Classification* (MUSIC) and RAP MUSIC provides precise localisations based on user defined assumptions. In Curry 6.0 software, there is also a solution called SWARM which combines LORETA and

In study III we used 64 channel recordings. Electrode position is ensured by use of a standardized hood. To estimate source localization the data were analyzed by use of the software package Curry 6.0. A priori we chose to analyze within the time-windows corresponding to the components of the butterfly plots which displayed all channels at a time the evoked potential. A standardised boundary element model (BEM) head model (Fuchs et al. 2002) was used, and the noise was user-defined from the pre-stimulus period. As all signal to noise ratios were relatively high (5-19), we could analyze the recordings individually, and the predominant source of each time-window (corresponding to a component) was described by the use of *Recursive-MUSIC algorithm* (Fuchs et al. 2004; Michel et al. 2004; Mosher & Leahy 1998). We displayed all coordinates in millimetres using the *Talairach* system (X: right (positive) / left (negative), Y: anterior (positive) / posterior (negative), and Z: up (positive) / down (negative)) (Talairach & Tournoux 1988). To determine an estimate of the number of sources in a given time window, we used principal component analysis, which decomposes the signal into a number of uncorrelated orthogonal patterns. We finalized the analysis by computing the data into an equal number of independent component analysis (ICA), which is believed to decompose the EEG into a number of independent component each having a single dominant source. Finally, we applied an independent component analysis-filter, which made it possible to model each of the contributing sources independently within a particular peak. We decided a priori that only sources which contributed with more than 10% and had a signal to noise ratio above 2 were accepted in the estimation procedure (Brown et al. 1979; Grech et al. 2008; Skrandies 1993).

In our dataset from study III, the predominant source contributed in all cases to the scalp potential with more than 59%. We found reproducible source locations in each of the four components (N1, P1, N2 and P2) in terms all three coordinates, x , y and z ($P=0.5$, $P=0.2$ and $P=0.8$ respectively). The confidence ellipsoid (expressed as ml) was between 8 and 57, and the residual

variance was between 5 and 31, being highest on the first peak (60 ms post-stimulus) immediately after DNIC induction.

As we wanted to model the brain activity, based on parallel dipoles, we computed the grand mean file of the 15 individual recordings. Then we modelled again by use of the same principles as described above. The rationale was to apply more than one dipole to the model. Hence the combination of the two methods could provide knowledge on the predominant dipole as well as parallel occurring brain activity in other brain areas. However, the major shortcomings of analysing a computed grand mean file is that the source estimate of the x, y and z-coordinates has no confidence interval and hence statistically differences in dipole locations can not be calculated. A further disadvantage is that grand mean files may contain signals, which cancel out when they are merged together or contain outliers which contaminate the overall solution. On the contrary as more dipoles contribute to the description of the surface potential the residual variance (the percentage of variance that is not described by the dipole model) will be smaller.

An aim of study III was to reconstruct the up-stream brain activation, which can elucidate the early pain-specific (exogenous) and later non-pain-specific (endogenous) responses (Hobson et al. 2005). Inverse modelling on CEPs following painful stimulation in the gut, based on BESA, was done by Dimcevski et al. The authors found dipolar activities corresponding to the early CEPs located in the bilateral insula, in the anterior cingulate gyrus and in the bilateral secondary somatosensory area (Dimcevski et al. 2007).

We chose to use dipolar sources estimates based on R-MUSIC combined with principal components analysis and independent component analysis to estimate the number of dipoles. MUSIC applied to this dataset showed to be stable and reproducible, see figure 16.

In study III, we found that induction of DNIC caused instant supra-spinal alterations brain responses, affecting the two endogenous components N2 and P2. The activity was observed in more frontal regions than the baseline activity primarily reflected in cingulate activity. Ten minutes after DNIC activation alterations were seen in the exogenous components N1 and P1. The findings likely reflect an activation of the frontal-limbic-brainstem top down inhibitory brain networks involved in the inhibitory pathways. The shift in predominant dipolar activity can be interpreted as a short-term cortical reorganisation, in which the inhibitory network is predominant.

Figure 16

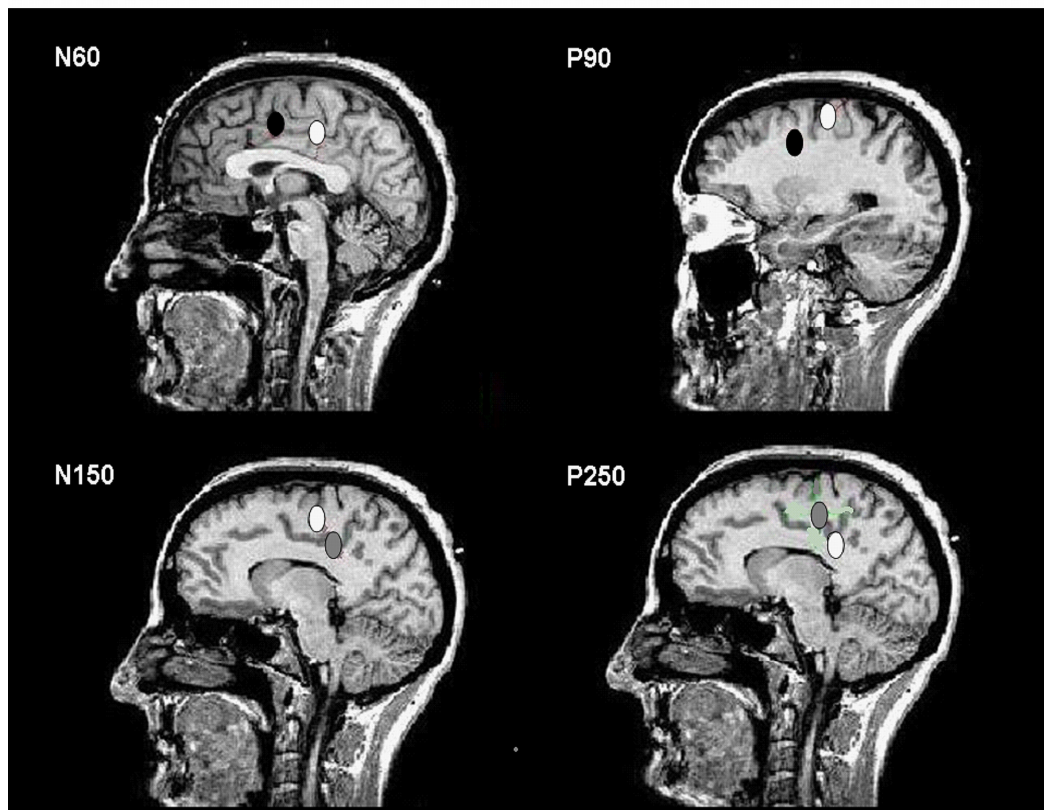


Figure 16: Dipolar reconstruction shows significant dipolar shifts due to physiologic reorganisation of brain activity after DNIC-activation. The baseline dipoles are shown to be reproducible. The dipolar sources that changed location immediately after the cold-pressor test are those appearing 150ms and 250 ms post-stimulus respectively. The reorganisation likely represents a response to endogenous modulation involving cognitive and affective processes. The dipolar sources that changed locations after 10 minutes are those appearing at 60ms and 90 ms post-stimulus representing the exogenous pain. The changed locations of these dipoles may correspond to an opening of the inhibitory network in the brain. The white dipoles indicate the baseline position, the grey dipoles indicate the dipole position immediately after DNIC activation, and the black dipoles indicate the dipole position 10 minutes after DNIC induction.

Table 3: The table provides an overview of different available techniques to solve the inverse problem, based on the following litteratur: (Fuchs, Ford, Sands, & Lew 2004; Grech R et al. 2009; Wagner, Fuchs, & Kastner 2007)

	Principles	Discussion
BESA <i>Brain elecyrical source analysis</i>	Estimates fixed dipole locations over a given time interval and then uses the whole block of data in the least square fit. The fitting results in a time-varying modulation of the amplitude (strength) of each of the dipoles.	The technique is very sensitive to the initial guess of the number of dipoles, and therefore it is highly dependent on the level of user expertise. The algorithms possess the drawback of possibly being "stuck in local minima". Over determination of too many sources.
s-LORETA <i>Standardized Low Resolution Electromagnetic Tomography</i>	Distribution source based on reconstruction of the brain electric activity in each point of a 3D grid of solution points, based on the idea that neighbouring neuronal populations are more likely (than non-neighbouring ones) to undergo synchronous depolarization. Selects a solution with a smooth spatial distribution.	Its outcome is not a distribution of currents modelling brain activity but a statistical map thereof. Hence, it is not the optimal tool for inverse modelling. Lower localization error than LORETA. If two areas with larger distances are correlated the algorithm produces blurred "over smoothed" solutions that can include two hemispheres. In non-noisy data it is found to have 100% accuracy.
SWARM <i>s-LORETA- weighted accurate minimum norm method</i>	Combines the techniques of diagonally weighted Minimum Norm Least Squares (MNLS) and s-LORETA to compute a current density vector field with low localization error. It is a solution to the inverse modelling	The algorithm is implemented in Curry 6.0 software and seems promising to use as a complementary tool to the existing algorithms. However, it has not yet been compared to other methods.
R-MUSIC <i>Recursiv Multiple signal classification</i>	Decomposes the data to identify the underlying components (signal space) in the time series. The signal-space is scanned for the optimal number of dipoles. Once they are found, the time course of each individual dipole is determined. An extension of the single equivalent current dipole is the <i>moving</i> dipole. Its three coordinates per timepoint is fitted, and its components are determined for each in a series of time.	Difficulties in distinguishing correlated sources. Decomposition overcomes the problem "stuck in a local minima". Shortcoming regarding correlated sources in the presence of noise
RAP-MUSIC <i>Recursively applied and projected Multiple signal classification</i>	Improvement of MUSIC	Provides an estimation of the minimum number of sources. Overcomes the problem of finding correlated sources encountered in MUSIC.

The dipolar source analysis mentioned above was applied to instantaneous CEP data. The disadvantage of modelling on instantaneous CEPs is the instability of algorithms when multiple sources are active and the interference of background electrical and physiological noise. For this reason, our group is currently developing different signal decomposition methods to be used in order to separate the signal into a sum of waveforms, each having a single dipole generator – hence, giving a qualitative description of the individual electrical source (Lelic et al. 2009). In the future, we are aiming at clustering the dipoles after source localization- based on time-frequency and localization. This procedure will be based on realistic head models for each individual (i.e. combining their individual MRI scans and digitized EEG electrode locations). These methods will allow for more precise source localization, automated separation of dominant sources during painful CEPs in different groups, and provide the ability to study the sequential order of activated centres post stimulus. These advancements will provide new insight into how different subject groups process pain.

CHAPTER IX

Conclusion and further perspectives

9.1 Development and reproducibility

We developed and evaluated a multimodal rectal probe, which could combine mechanical, electrical and thermal stimulation. The psychophysical assessments perceived from these stimulations were highly reproducible in healthy volunteers (study I) both between study days and between individuals. Therefore we integrated the probe in a platform designed to assess viscerovisceral hyperalgesia (study II) in sensitized volunteers. We used the multi modal rectal probe for testing the influence of DNIC on sequential real-time brain activity in healthy volunteers (study III). The probe is currently being used to examine: 1) altered rectal sensations in patients suffering from chronic pancreatitis before and after pregabalin treatment; 2) altered rectal sensation in patients suffering from IBS before and after temporary sacral nerve stimulation; 3) altered rectal sensation in patients suffering from megarectum and 4) altered rectal sensation of healthy volunteers before and after pharmacological interventions. Future studies include 1) a multi-segmental, multi-tissue study in patients suffering from diabetes mellitus and 2) a study investigating faecal evacuatory disorders.

9.2 The viscerovisceral extra-segmental model

We established a multimodal (study I) and multi-segmental model, in which we had access to both oesophagus and rectum (study II). The aim was to assess hyperalgesia in the oesophagus (8 cm proximal to the perfusion site) as a proxy of secondary hyperalgesia/allodynia. We found a higher threshold to electrical stimulation and diminished referred pain areas. Thus, we conclude that the chemical sensitization (most likely through the capsaicin-component) induced an activation of descending inhibition. The sensory manifestations in the rectosigmoid and the rectum were also altered after the sensitization. As in the oesophagus, we observed heightened pain detection threshold for electrical stimulations. On the other hand, both mechanical and heat stimulation of the rectum, showed decreased pain detection threshold. Thus we conclude that the combined acid+capsaicin perfusion induced modality-specific extra-segmental visceral hyper-/hypoalgesia. The model mimics the clinical pain to a higher degree, because both inhibitory and facilitatory (central sensitization) mechanisms are brought into play.

9.3 Latencies and amplitudes of evoked brain potentials after pain-modulation

We analyzed evoked brain potentials in terms of latencies and amplitudes and were able to find the four reproducible well defined peaks, which appeared before 350 ms post-stimulus. *Experimental sensitization* decreased the latency for two of the components, which likely reflects opening of latent and parallel pathways in pain processing (study II). The amplitude reflects the synchronous neuronal activity, and does not necessarily reflect pain perception.

Induction of DNIC had no immediate influence, neither on the latencies nor on the amplitudes. However, we saw increases in latency, amplitude and dipole strength 10 minutes after DNIC induction. Hence, we believe it indicates a rebound effect suggesting parallel endogenous processing in the inhibitory brain network (study III).

9.4 Brain activity to painful recto-sigmoid stimuli after DNIC induction

Dipolar source modelling showed instant alterations of the endogenous brain-responses to the painful stimuli, which indicate activation of supra-spinal inhibitory networks (study III). The predominant endogenous pain processing in the cingulate cortex, suggests this structure as a coordinating role in the frontal-cortico-limbic-brainstem top-down inhibitory network, which is believed to modulate the cognitive and affective components of pain. Ten minutes after DNIC induction dipolar source modelling showed alterations in the ascending up-stream exogenous brain-responses. Brain areas such as prefrontal cortices, supplementary motor area and amygdale are known to possess high density of inhibitory receptors. Hence, we believe that the short-term reorganisation with predominant electrical activity in the more frontal brain regions may reflect a communication between top-down inhibitory control and DNIC. We believe that the findings in this study indicate that the exogenous pain perception is influenced by the inhibitory network minutes after the conditioning stimuli (DNIC induction by cold-pressor) and that endogenous modulation is modulated instantly.

9.5 Future perspectives

The novel multimodal rectal and rectosigmoid sensory testing approach has been developed and evaluated. The probe has already been integrated in protocols investigating functional pathophysiology in patients with pain and hyper/hypo sensitivity in the large intestine. Currently the probe is used in a study investigating patients with chronic pancreatitis and in patients suffering from IBS treated with temporary sacral nerve stimulation. Furthermore it is used in two studies to assess pharmacological interventions in healthy volunteers and patients. As the probe is easy to integrate in more advanced experimental models, the novel developed multimodal and multi-segmental model provides new options for further investigations in chronic pain patients where the descending control is impaired. The possibility of exploring real-time brain activity of the ascending sensory pathways provides a unique approach to study basic pain mechanisms and pharmacologic interventions in both healthy subjects and in patients exhibiting impaired descending control (such as those observed in several functional and organic gastrointestinal disorders).

We are currently working on a protocol, which have an integrated approach including 1) quality of life (SF36) 2) autonomic, 3) neuroendocrine, 4) psychophysical, 5) pain-modulatory and 6) neurophysiological assessments in a multi-centric (Denmark, Sweden, Hungary and Norway) protocol *DIAMARK*, which have received EU-funding. We believe that changes in the neuronal pain matrix with

interactions between peripheral and central pain mechanisms are likely to be involved in the pathogenesis of gastrointestinal symptoms in long-standing diabetes (Frokjaer et al. 2007). Hence the aim is to develop biomarkers for detection of autonomous neuropathy as a result of long-term altered glucose-metabolism in patients suffering from diabetes mellitus. Pain assessments are based on multi-tissue (skin, muscle and viscera) multi-segmental (duodenum, oesophagus and rectum) and multi-modal stimulations (electrical, thermal, mechanical). In the neurophysiological approach descending inhibition investigated with EEG recordings of 128 channels and evoked potentials will be analyzed. To optimize the use of a multi-channel recordings, the accurate electrode position combined with individual MR scans will be applied, as especially the thickness of the skull distort or attenuate the surface recordings. Analysis of latencies, amplitudes and source locations will be applied. The recently developed Multichannel Matching Pursuit (MMP) will be used, which decomposes the data into a sum of waveforms (usually termed atoms), each of them defined in time, frequency and space. We have showed that decomposing the evoked brain potentials using MMP prior to inverse modelling (using MUSIC) is superior to some blind source separation methods such as Independent Component Analysis and Second-Order Blind Identification. Currently, the group is developing a toolbox to cluster MMP atoms based on time/frequency, topography, both time/frequency and topography, or dipoles. We believe that these new approaches, will lead us in the direction of the ultimate pain model, in which we aim at being able to follow the ascending pain (and hopefully descending) coding signal in real-time from the spinal level to brainstem and supraspinal centres.

Danish summary:

Udvikling af nye smerte-modeller til at stimulere og modulere eksperimentel smerte i endetarmen hos raske forsøgspersoner, baseret på subjektive smerte mål og objektiv neurofysiologi.

Ph.D-afhandlingen er baseret på tre originalarbejder. Formålet har været at udvikle og afprøve nye metoder til human eksperimentel smerteforskning af mavetarmkanalens sensoriske nervesystem.

Mavesmerter er blandt de mest almindelige symptomer, når patienter søger lægehjælp. De grundlæggende mekanismer bag disse mavesmerter er dårligt belyst, og derfor er behandlingsmulighederne få og ofte utilstrækkelige. En af måderne man kan forbedre viden på området, således at patienter i fremtiden kan tilbydes en bedre smertebehandling, er ved at øge forståelsen for de underliggende mekanismer. Derfor er det vigtigt at undersøge og klarlægge de nervebaner som fører føle- og smerte information fra spiserør, endetarm og den bageste del af tyktarmen videre til rygmarven og hjernen. Resultaterne kan herefter bruges i basale, kliniske og farmakologiske studier. Smertegruppen Mech-sense har tidligere positive erfaringer i at udvikle en smerte-model til spiserøret. På baggrund af disse var målet at etablere en lignende model til den nedre del af tarmen. Den nyudviklede metode er baseret på multimodal stimulation, det vil sige stimulering af tarmen med flere forskellige standardiserede smertestimuli: elektriske impulser, varme, kulde og mekanisk stræk. Således aktiveres flere af de receptorer og smertebaner, der ligeledes aktiveres under kliniske sygdomme. Metoden er nu internationalt anerkendt, og bruges til undersøgelse af patienter i Århus, bergen og London.

Store dele af befolkningen rammes af sygdomme, der giver anledning til symptomer som luft i maven, vekslende afføringsmønster og kroniske mavesmerter. Ofte beskrives årsagen til sygdommen som øget følsomhed i tarmvæggen, hvilket giver sig til udtryk som øget smerte respons. Følenervene fra et tarm-segment påvirker følenervene fra et andet tarm-segment i rygmarven. Dette kaldes central sensibilisering. På den måde kan følsomheden i et i øvrigt rask organ ændres, fordi et andet indre organ har en smertevoldende tilstand. Den lægelige vurdering af patientens smerter kompliceres yderligere af at patientens symptomer er en balance mellem smerter og kroppens evne (eller manglende evne) til at kontrollere smerter.

Vi etablerede dernæst en udvidet model som forsøger at efterligne den kliniske situation, for på den måde at opnå indsigt i den basale patofysiologi, der ligger bag central sensibilisering ved forskellige sygdomme. Således målte vi følsomheden i endetarmen og den bageste del af tyktarmen, efter at have fremkaldt en lokal irritation (bestående af saltsyre og chili) af spiserøret.. Ved at sammenholde de subjektive smerte mål med hjernens bearbejdning af smerten, kan nogle de komplekse ændringer, som foregår i centralnervesystemet, belyses.

Hjernen og rygmarven kontrollerer kroppens egen evne til smertekontrol, og nedsat eller dårlig smerte-kontrol, er medvirkende årsag til kroniske smerter.

Vi udviklede en model for at belyse hvilke hjerne-områder, som aktiveres under smertekontrol. Denne nye viden kan bruges i fremtiden til at undersøge hjernens bearbejdning hos kroniske smerte patienterne, som helt eller delvist mangler smertekontrollen.

Konkluderende kan man sammenfatte at de nyudviklede multimodale og multisegmentale metoder, vil kunne bidrage væsentligt til den fremtidige forståelse af patofysiologien og behandlingsstrategien ved komplicerede smerterelaterede lidelser i mavetarmkanalen. Metoden vil kunne anvendes til at nærme sig en mere fuldstændig forståelse af interaktionen mellem receptorer, tarm, nerve, rygmarg og hjerne.

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